AFRL-RH-FS-TR-2013-0004

A Comparative Study of Melanin Content and Skin Morphology for Three Commonly Used Laboratory Swine (*Sus scrofa domestica*)

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September 2012

Final Report for May 2009 to May 2011

Air Force Research Laboratory
711th Human Performance Wing
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REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE *(DD-MM-YYYY)*	**2. REPORT TYPE**	**3. DATES COVERED** *(From - To)*
09-19-2012	Final Technical Report	May 2009- May 2011

4. TITLE AND SUBTITLE
A Comparative Study of Melanin Content and Skin Morphology for Three Commonly Used Laboratory Swine (*Sus scrofa domestica*)

5a. CONTRACT NUMBER
FA8650-08-D-6930

5b. GRANT NUMBER

5c. PROGRAM ELEMENT NUMBER
0602202F

6. AUTHOR(S)
Rebecca Vincelette, Aurora Shingledecker, Robert W. Kornegay, Rick Figueroa, Dawnlee Roberson, Katharine E. Sheldon, Jeffrey Oliver, Carist Washington, and Nichole Jindra

5d. PROJECT NUMBER
7757

5e. TASK NUMBER B2

5f. WORK UNIT NUMBER 39

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
Air Force Research Laboratory
711th Human Performance Wing
Human Effectiveness Directorate
Bioeffects Division
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8. PERFORMING ORGANIZATION REPORT

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
Air Force Research Laboratory
711th Human Performance Wing
Human Effectiveness Directorate
Bioeffects Division
Optical Radiation Bioeffects
Fort Sam Houston, Texas 78234

10. SPONSOR/MONITOR'S ACRONYM(S)
711 HPW/RHDO

11. SPONSOR/MONITOR'S REPORT NUMBER(S)
AFRL-RH-FS-TR-2013-0004

12. DISTRIBUTION / AVAILABILITY STATEMENT
Distribution A: Approved for public release; distribution unlimited (approval given by local Public Affairs Office TSRL-PA-13-0027)

13. SUPPLEMENTARY NOTES

14. ABSTRACT

Yorkshire and Yucatan Mini-pig breeds have been commonly used for laser-tissue studies. The newer Yucatan Micro-pig breed has not been used in laser-tissue studies, but could be an alternative to meet logistical needs for laser-tissue studies if its skin is comparable to the accepted Yorkshire and Yucatan Mini-pig breeds. This study sought to contrast the differences in skin thickness and melanin content in pig skin across domestic swine breeds. Skin thickness was measured using H&E stained slides while the melanin index (ratio of melanin area to a selected area of tissue) was measured using a Fontana-Masson stain. Six biopsies were acquired from the flanks of each subject; eight Yorkshire pigs, two Yucatan Mini-pigs, and one Yucatan Micro-pig. Statistically significant intra-breed variations in the thicknesses of the stratum corneum, epidermis and dermis were found between equivalent sample sizes in the Yorkshire group. The Yucatan Micro-pig was found to have skin thicknesses more similar to the Yucatan Mini-pig's compared to the Yorkshire. Results for the melanin index values indicate that the Yorkshire pig breed is most likely to have an average melanin index near 0.37%, with the Yucatan Mini- and Micro-pigs trending towards melanin index values greater than that of the Yorkshire pig breed. The sample sizes for the Mini- and Micro-pigs were insufficient to assess the complete melanin index values between breeds. Though this is not an equivalent sample size comparison, results indicate that the skin thickness and melanin distribution in the new Micro-pig are more similar to the Yucatan Mini-pig and not the Yorkshire breed, suggesting the Micro-pig may be a suitable substitute for experiments which have traditionally used the Mini-pig.

15. SUBJECT TERMS

16. SECURITY CLASSIFICATION OF:			**17. LIMITATION OF ABSTRACT**	**18. NUMBER OF PAGES**	**19a. NAME OF RESPONSIBLE PERSON**
a. REPORT	**b. ABSTRACT**	**c. THIS PAGE**			**19b. TELEPHONE NUMBER** *(include area code)*
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ABSTRACT

Yorkshire and Yucatan Mini-pig breeds have been commonly used for laser-tissue studies. The newer Yucatan Micro-pig breed has not been used in laser-tissue studies, but could be an alternative to meet logistical needs for laser-tissue studies if its skin is comparable to the accepted Yorkshire and Yucatan Mini-pig breeds. This study sought to contrast the differences in skin thickness and melanin content in pig skin across domestic swine breeds. Skin thickness was measured using H&E stained slides while the melanin index (ratio of melanin area to a selected area of tissue) was measured using a Fontana-Masson stain. Six biopsies were acquired from the flanks of each subject; eight Yorkshire pigs, two Yucatan Mini-pigs, and one Yucatan Micro-pig. Statistically significant intra-breed variations in the thicknesses of the stratum corneum, epidermis and dermis were found between equivalent sample sizes in the Yorkshire group. The Yucatan Micro-pig was found to have skin thicknesses more similar to the Yucatan Mini-pig's compared to the Yorkshire. Results for the melanin index values indicate that the Yorkshire pig breed is most likely to have an average melanin index near 0.37%, with the Yucatan Mini- and Micro-pigs trending towards melanin index values greater than that of the Yorkshire pig breed. The sample sizes for the Mini- and Micro-pigs were insufficient to assess the complete melanin index values between breeds. Though this is not an equivalent sample size comparison, results indicate that the skin thickness and melanin distribution in the new Micro-pig are more similar to the Yucatan Mini-pig and not the Yorkshire breed, suggesting the Micro-pig may be a suitable substitute for experiments which have traditionally used the Mini-pig.

1 INTRODUCTION

A main focus of the Air Force Research Laboratory (AFRL) 711th Human Performance Wing (711 HPW), Human Effectiveness Directorate, Bioeffects Division, Optical Radiation Bioeffects Branch (RHDO) is to study the bioeffects incurred as a result of irradiation from an exposure to laser beams. To determine how light interacts with tissue, many experiments require biopsies of laser irradiation sites to analyze the extent and type of damage through histological observation (Rico *et al*., 2000, Eggleston *et al*., 2000, Niemz, 2007, Welch & van Gemert, 2011). Previous skin comparison analysis has been performed on Yorkshire and Yucatan Mini-pigs to verify their use as a human substitute for use in the field of photonics (Eggleston *et al*., 2000).

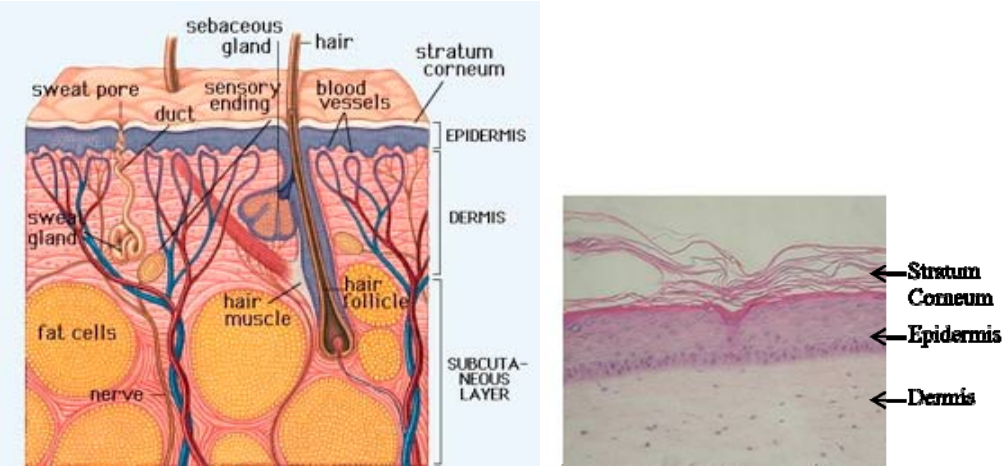
Morphological examination of tissue is important for both the experimental and theoretical research efforts involving laser-tissue interaction. On the experimental side, such evaluations lend evidence toward proper selection of an animal model (*in vivo*) or for tissue constructs (*in vitro*). On the theoretical side, data can be used to construct more accurate *in silico* tissue models.

This study compared histological data from swine breeds typically used in skin damage studies (Yorkshire and Yucatan Mini-pig) to the recently introduced Yucatan Micro-pig. The Micro-pig was created through selective breeding of the Yucatan Mini-pig, and yields adult pigs in the 20 - 35 kg range (Sinclair Farms). The Yucatan Micro-pig has been used for skin permeation studies, but not for laser-safety studies within AFRL (Peter, *et al.*, 2001; Lehmann, 1998; Pak, *et al.* 2005).

Presently there are little to no data assessing skin thickness measurements or melanin content of the Micro-pig's skin. Eggleston *et al*. (2000) compared the skin of the Yorkshire and Yucatan Mini-pig breeds, reporting that the Yucatan is better suited for laser-skin studies than the Yorkshire breed due to its similarities to human skin. If the skin of the Yucatan Micro-pig is similar to the Yucatan Mini-pig, then the Micro-pig would be a suitable breed substitute for laser-tissue experiments that typically use the Mini-pig. Our hypothesis is that the skin from the Yucatan Micro-pig will have skin depth and melanin concentration values more similar to the Yucatan Mini-pig compared to Yorkshire breeds.

1.1 General Histology of the Skin

The skin is an inhomogeneous organ composed of several layers (Figure 1).



(A) (B)

Figure 1. Basic structure of the skin.
(A) Colored drawing (figure from http://skinsynergy.wordpress.com/basic-knowledge-skin-structure/).
(B) How a standard cross section of the skin (H & E stained) appears under a light microscope (figure adapted from http://www.mattek.com/pages/products/epidermft/)

The outer-most layer of the skin is the stratum corneum which consists of mostly dead cells called corneocytes (Agache, 2004). This layer is commonly considered to be part of the epidermis (Agache, 2004 and Krishnaswamy & Baranoski, 2004), but provides one critical function as a barrier between the mammalian organism and the environment. On humans, the stratum corneum ranges in thickness from approximately 8 to 20 μm, but is about ten-times thicker on the palms and soles (Holbrook & Odland, 1974). Corneocytes are held together by a lipid-based intracellular mixture which covalently bonds with proteins on the cell membrane (Agache, 2004). The outermost surface of this layer gradually sloughs off and is replenished with new corneocytes. In humans, the stratum corneum is completely replaced approximately every 6 to 26 days (Agache, 2004). Water content in this layer of the skin follows a gradient with less water at the surface (5-10%), but is dependent upon the environmental conditions (i.e., dry air, high humidity, etc.) at the skin's surface (Agache, 2004). The stratum corneum absorbs very little optical radiation (Everett *et al*., 1966).

Below the stratum corneum resides the epidermis. In humans, the epidermis ranges in thickness from 50-100 μm depending on anatomical location, except for the palms and soles, where thicknesses can reach 1 mm (Gentilhomme and Neveux, 2004). Though the epidermal layer is composed of approximately only 10 layers of cells, called keratinocytes, it provides numerous important functions for maintaining the skin organ. Functions of the epidermis are to generate the stratum corneum, hormone secretion, cytokine production for angiogenesis, and vasomotricity in the papillary dermis, homing and maturation of cells responsible for the immune barrier, protection against ultraviolet

light and homing of melanocytes, participation in the skin neurosensorial function and homing of Merkel cells, participation in the skin mechanical protective function and self repair (Gentilhomme and Neveux, 2004). After the stratum corneum layer, there are four sub-layers in the epidermis; stratum lucidum, stratum granulosum, stratum spinosum, and stratum basale (Krishnaswamy & Baranoski, 2004).

Stratum lucidum is the translucent area just below the stratum corneum. This layer is typically only observed under the light microscope in thicker skin, like on the palms or soles of the hands and feet (http://medical-dictionary.thefreedictionary.com/stratum+lucidum). The stratum granulosum layer consists of 3-5 layers of flattened cells with deteriorating organelles. The stratum spinosum layer consists of several layers of keratinocytes held together by desmosomes. The deepest epidermal layer is the stratum basale and has a single layer of active mitotic stem cells, some which form into melanocytes. The melanocytes are found in the basale layer of the epidermis and are responsible for producing melanin. Melanin is of particular importance for laser-tissue interaction as it plays a significant role in the absorption of optical radiation (Welch and van Gemert, 2011). Melanin is found in organelles called melanosomes, which appear as granules around 500 nm in size when observed with the light microscope (Wasmeier *et al*., 2008).

There are two major types of melanin: eumelanin (brown-to-black pigments) and pheomelanin (red-to-yellow pigments) (Simon *et al*., 2008 and Thody *et al*., 1991). The two major types are classed based on their molecular pathways in melanogenisis (Simon *et al*., 2008). Melanin is believed to have evolved as a photoprotection mechanism from UV radiation, where it converts this radiation into heat and prevents absorption by other body components. Melanin granules are often spindle shaped, as opposed to round, and absorb optical radiation throughout the whole volume of the granule (Wolbarsht *et al*., 1981). Water molecules are believed to influence optical properties of the melanin granule (Bridelli & Crippa, 1981). Melanosomes have been reported to occupy 1.3 to 43% (volume fraction) for light-to-dark pigmented skin in the epidermis (Jacques, 1996). The melanocytes produce dendrites, extending into the intracellular space in the basale layer. There are several theories as to how melanosomes move from the melanocyte into neighboring cells (Slominski *et al*., 2004). One popular theory is the melanosomes move into the dendrites and migrate onto the outer edge of the melanocyte where they can be phagocytized by neighboring keratinocytes (Leroy, 2004, Pg. 475). Note that keratinocytes produce keratin, the primary constituent for hair. Melanosomes enter the hair when it is being formed beneath the skin's surface.

The production of melanin, called melanogenesis, is regulated by a complex molecular pathway. Melanogenesis is sensitive to UV radiation, endothelines, histamine, eicosanoids, sex steroids, and vitamin D (Slominski *et al*., 2004). The role of sex steroids (i.e. estrogen) is complex and has been demonstrated to both promote and inhibit melanogenesis depending on unknown factors (Thorton, 2002). Though light can be absorbed by melanosomes in the epidermis and hair, optical radiation can still penetrate through this layer of the skin into the dermal layer below.

The dermal layer of the skin consists of two sub-layers: papillary and reticular dermis (Agache, 2004, Pg. 199). The papillary layer, also referred to as the subpapillary and advential dermis, is approximately 20-100 μm thick in humans, while the reticular dermis, also referred to as the chorion, is about 10-20 times thicker (> 500 μm thick). The papillary dermis is composed of a network of collagen and elastin fibers, loosely woven together such that immune cells (i.e., phagocytes) can move into this layer to respond to bacteria and/or other foreign bodies, which could penetrate the skin. The reticular dermis resides below the papillary layer and is composed of a dense fibrous network of tissues, which serve as a solid, compressible mechanical support structure for the skin organ (Agache, 2004). Blood vessels can be found in the dermis, with larger vessels occupying the reticular layer compared to the papillary layer. Blood is also known to absorb optical radiation, thus contributing to the overall thermal response for some optical radiation wavelengths. Below the dermis is the subcutaneous tissue, also called the hypodermis. This layer is composed primarily of fat cells and interstitial tissue to hold the skin to the underlying tissues of the body (Agache, 2004).

1.2 Experimental Skin Models

Domestic pig skin has both morphological and functional characteristics similar and dissimilar to human skin (listed in Table 1) (Meyer *et al*., 1978). While many of these characteristics are not directly important for the results of a laser exposure, they may contribute to the exposure response indirectly. Within the domestic pig family, *Sus scrofa domestica,* there are numerous breeds, each of which has its own skin characteristics. A basic literature search reveals that pigs are used extensively for research, where topics specifically involving skin include wound healing, dermal and transdermal toxicology, and carbohydrate metabolism in the skin (Swindle and Smith, 1998). Each of these research efforts requires detailed knowledge about the skin to validate a particular breed of swine skin selected for the experiment. For research in laser-skin exposure, the important parameters are those that affect laser absorption leading to a thermal response. Melanin concentration is an important element when examining laser-tissue interaction due to its high absorption coefficient in the visible range. Thus it is important to examine melanin distribution in pig breeds.

Table 1. Similarities and differences of pig skin to human skin

Similarities	Dissimilarities
Sparse haired coat	Poor vascularization of cutaneous glands
Thick epidermis with distinct rete pegs and dermal papillae	Absence of eccrine cutaneous glands
High concentration of elastic fibers in dermis	Extensive fat deposits below subcutis
Vasculization of hair follicles	Seasonally regulated hair shedding
Structure of the collagenous tissue framework	Apocrine skin glands not involved in thermoregulation
Epidermal tissue turnover time	

To examine skin, histologically prepared samples are stained to increase contrast for examination under the light microscope. The most common stain is hematoxylin and eosin (H&E) -- which is not designed to stain melanin in the skin -- and has been used to determine only the distribution of melanin in the epidermis (Eggleston *et al*., 2000). A recent study on the melanin distribution in pigmented hairless guinea pigs used Steiner Stain, a melanin-specific stain, to improve the visibility of the melanin granules under the light microscope (Jindra & Imholte, 2009). When Jindra and Imholte compared Steiner-stained slides to slides using the H&E stain, they observed a higher number of melanin granules compared to the Steiner-stained slides. Thus, data currently available for use in modeling photon interaction with melanin may be too limiting, resulting in overly conservative safety standards and thereby restricting personnel and lasers in critical operations.

Unfortunately, the Steiner stain has a higher contrast for all organelles in the cell, making it difficult to automate the counting process because the computer cannot differentiate between melanin and other highly stained cells. Previous work has reported melanin concentrations (Eggleston *et al*., 2000), but this study focused on the quantity of melanin in order to generate a melanin concentration index (where melanin index is the ratio of melanin area to a selected area of tissue). This required that we generate an image with a high contrast for melanin only to automate melanin counting. The Fontana-Masson stain allows for this, as the melanin reduces the stain to a silver color, which is readily detectable.

The goals of this study were to quantify the amount of melanin in the skin in each breed of swine in addition to measuring the thickness of the stratum corneum, epidermis, and dermis of the skin in each breed of swine and contrast to previous reports.

2 MATERIALS AND METHODS

The animal use protocol for this study was approved by the Institutional Animal Care and Use Committee at Brooks City-Base. All subjects were part of a tissue-sharing program with other investigators, so no subjects were utilized exclusively by this work.

2.1 Experimental Skin Samples

This project utilized skin from the flanks of 11 animals: 8 male Yorkshires, 2 female Yucatan Mini-pigs, and 1 Yucatan Micro-pig of unknown gender. Subject's flanks were examined for any aberrations prior to skin collection. A 5-mm biopsy punch was used to remove three sections of skin from each subject's left and right flanks – a total of six samples per subject, resulting in 48, 12 and 6 biopsy samples from each breed of Yorkshire, Yucatan Mini-, and Yucatan Micro-pig respectively. Each sample was placed into a pre-labeled holder and then inserted into a jar of 10% neutral buffered formalin, and transferred for biopsy. Samples were then sent to a pathologist for assessing the skin thickness and melanin content.

2.1.1 Histology.

Biopsy cores were bisected through the skin-dermis-subcutis axis and processed together into a paraffin-embedded block using standard methods. Four-micron-thick sections were cut on a microtome, attached to a glass slide, de-paraffinized, and stained. Samples used for skin thickness measurements were stained with Hematoxylin-Eosin (H&E). Samples used for melanin index measurements were stained using Fontana-Masson (F-M).

Measurements were made using a computer-assisted image analysis software program (Image Pro-Plus Version 7.0, Media Cybernetics, Inc, Bethesda, MD 20814). Images were captured using an Olympus DP 70 Microscope Digital Camera with associated proprietary software (Olympus America, Inc, Melville, NY 11747-3157, USA) attached to an Olympus AH-2 microscope. Spatial calibration of the software for each objective magnification was done using an Olympus Objective Micrometer. Figure 2 is a typical section.

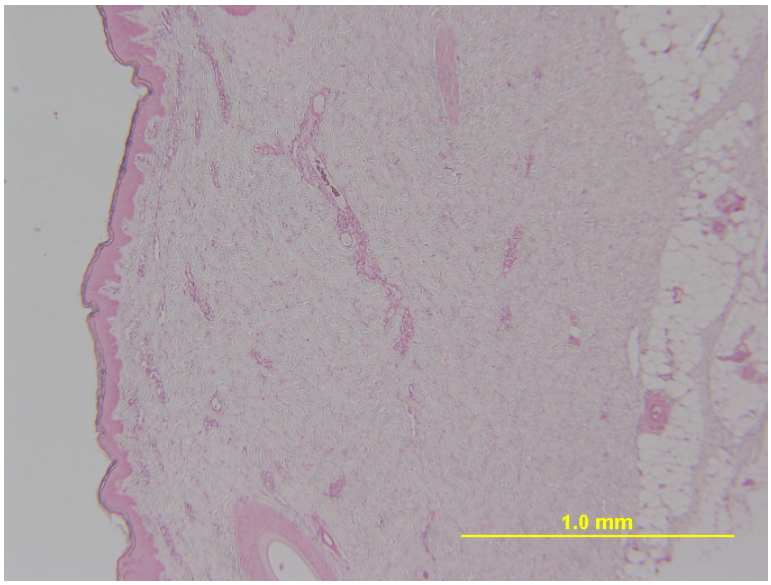


Figure 2. A typical skin histological section image from swine

2.1.2 Measuring Skin Thickness.

Two tissue sections (mirror images) from the bisected skin biopsy were usually available on each slide. A coin was tossed to determine which section would be imaged. If only one section could be adequately visualized, that section was used. A 4080 x 3072 pixel image was captured using a 2X objective. The section was aligned so that the epidermal surface of the skin was approximately perpendicular to the long axis of the image. A second coin was tossed to determine if the mouse pointer would be aligned with the upper or lower junction of the dermis and subcutaneous tissue in the captured image. The location on the vertical axis of the pixel map was noted. To generate a random measuring point, a single die was tossed, and then the count on the die was multiplied by 100. This number was added/subtracted from the vertical axis of the pixel map to obtain the first measuring point. One hundred pixels were then added (down from lower junction) or subtracted (subtracted from upper junction) as appropriate to obtain each of 10 measuring points. Using the image analysis software, the width of the dermis in microns was measured across the tissue from the starting point along the horizontal axis of the pixel map from the stratum basalis to the first subcutaneous tissue reached. Zooming the image allowed measurement of the epidermis (without stratum corneum) and the stratum corneum in microns. Starting at the point where each of the 10 dermal measurement lines intersected with the stratum basalis, the width of the epidermis (without stratum corneum) was measured along a line approximately perpendicular to and reaching the base of the stratum corneum. Similarly, the width of the stratum corneum was measured along a line approximately perpendicular to and reaching the surface or first point of separation within the stratum corneum.

2.1.3 Measuring Melanin Index.

Two tissue sections (mirror images) from the bisected skin biopsy were usually available on each slide. A coin was tossed to determine which section would be imaged. If only one section could be adequately visualized, that section was used. The section was aligned so that the epidermal surface of the skin was approximately perpendicular to the long axis of the image. A second coin was tossed to determine if the mouse pointer

would be placed over the upper or lower end of the epithelium in the selected section. The microscope stage was moved (upward or downward) the number of fields corresponding to the resulting number on a cast die. A 4080 x 3072 pixel image of this field was captured using the 40X objective and analyzed. A field was skipped and another image captured and processed. The process was repeated until 3 microscopic fields from each section were analyzed.

Using the image analysis software, a region of interest (ROI) was outlined for analysis which enclosed the entire area of the epidermis located in the microscopic field. The ROI was "segmented" to isolate the area of melanin staining gray-scale pixel values. The area of melanin was counted as measured in square microns. The image was then segmented again to count the entire area within the ROI in square microns. An example of how melanin index was measured is presented in Appendix 1.

The area of melanin was divided by the total area of the ROI to obtain a melanin content index. The melanin content index was multiplied by 100 to obtain the percentage of the area occupied by melanin (melanin % epidermal area).

3 RESULTS

The Yucatan Mini-pig and Yorkshire skin thickness and melanin skin models have been previously reported (Eggleston *et al*. 2000), but the Yucatan Micro-pig has not. The structure of the skin of the Yucatan Micro-pig and Mini-pig appear to be similar, as shown in Figure 3. All have similar epidermal strata with irregular patterns of dermal papillae and epidermal pegs, as previously reported (Eggleston *et al*. 2000). Data from only one Yucatan Micro-pig were able to be obtained, so the comparison is limited.

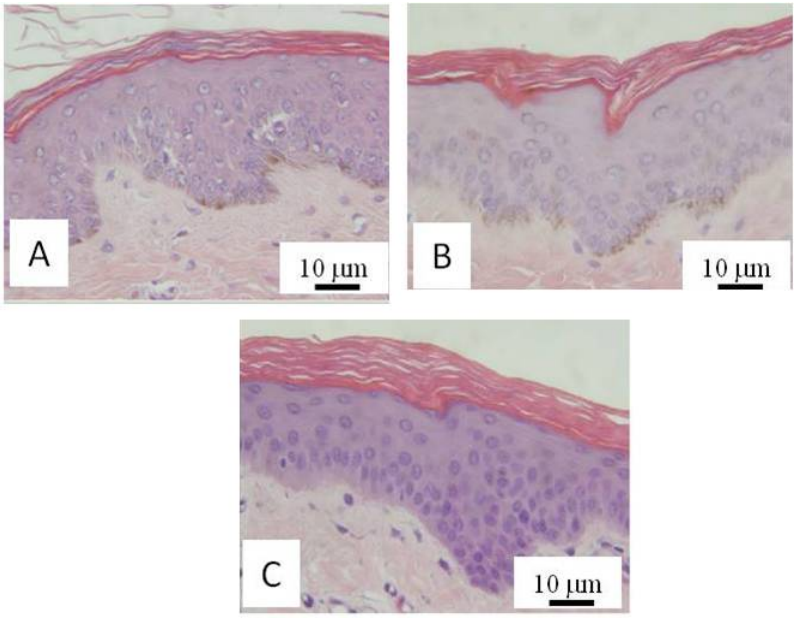


Figure 3. Comparative cross sections of A) Yucatan Micro-pig, B) Yucatan Mini-pig and C) Yorkshire

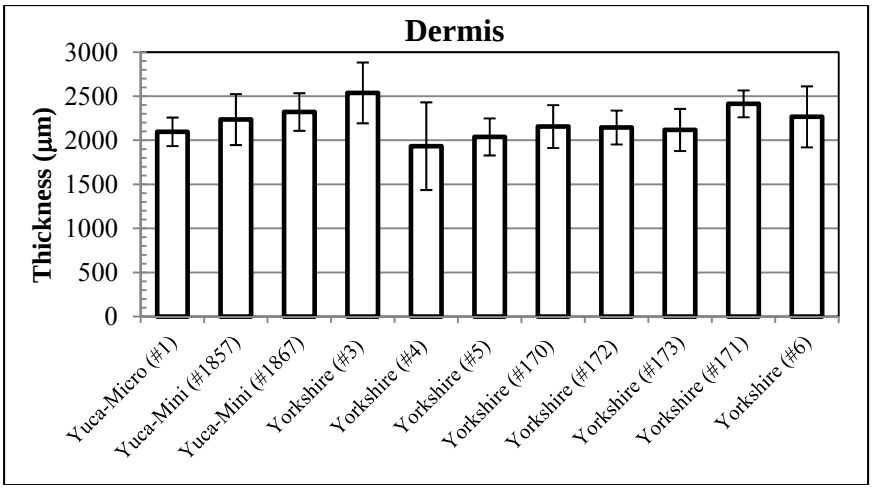
3.1 Skin Thickness

The raw histological data from individual subjects by slide number are reported in Appendix 4. Each slide number and section letter came from a single biopsy. There were six biopsies acquired per subject. The dermis, epidermis, and stratum corneum skin thickness was measured a total of 10 times per section letter. Thus data represented in Table 2 were averaged for each layer using a sample number of 60 (6 biopsies * 10 measurements per layer) per each subject number.

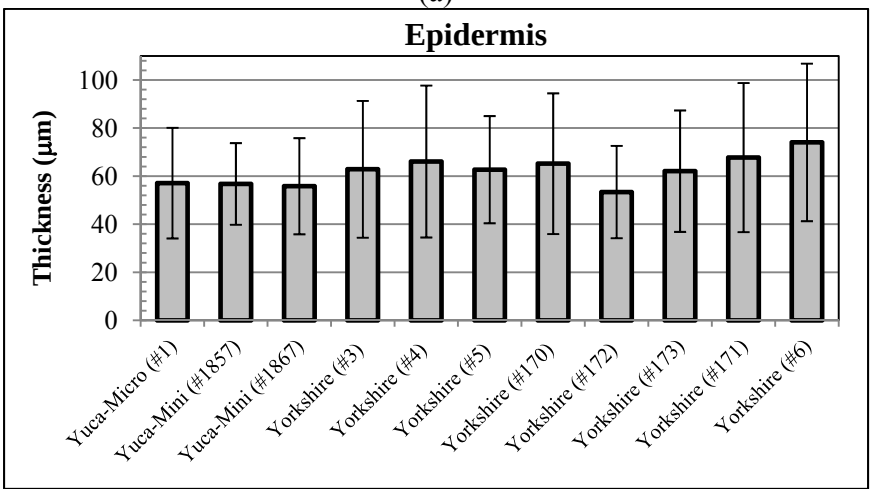
Table 2. Averaged skin thickness results by subject number. Each subject number had a total of 60 thickness measurements per layer

		Average Thickness (μm)			Standard Deviation (+/- μm)		
Slide # (Sections)	Breed (Subject #)	Dermis	Epidermis	Stratum Corneum	Dermis	Epidermis	Stratum Corneum
235 (A-F)	Yuca-Micro (#1)	2096.69	57.10	24.31	161.74	23.01	8.80
57 (A-C) & 58 (A-C)	Yuca-Mini (#1857)	2235.48	56.76	20.82	288.74	16.99	9.11
59 (A-C) & 60 (A-C)	Yuca-Mini (#1867)	2321.20	55.81	25.39	213.18	20.01	11.86
236 (A-F)	Yorkshire (#3)	2538.03	62.86	31.18	344.95	28.46	12.09
237 (A-F)	Yorkshire (#4)	1933.52	66.09	33.43	497.19	31.59	12.61
238 (A-F)	Yorkshire (#5)	2038.12	62.71	23.18	210.13	22.31	6.78
239 (A-F)	Yorkshire (#170)	2155.99	65.20	30.84	243.38	29.27	10.74
240 (A-F)	Yorkshire (#172)	2145.30	53.39	27.26	192.30	19.20	10.23
241 (A-F)	Yorkshire (#173)	2117.63	62.07	25.71	239.34	25.26	5.44
242 (A-F)	Yorkshire (#171)	2413.84	67.74	30.57	152.58	31.01	9.69
243 (A-F)	Yorkshire (#6)	2266.32	74.04	26.97	346.43	32.78	11.52

A graphical depiction of the skin thicknesses by subject are shown in Figure 4. An F-test was used to determine if data between subjects had equal variances. If the p-value was $\leq$ 0.05, then data between subjects were treated as having different variances. A student's t-test (two-tailed with equal or unequal variance based on the F-test results) was then run between each subject. Results from the F-test and t-tests between subjects are reported in Appendix 3.



(a)



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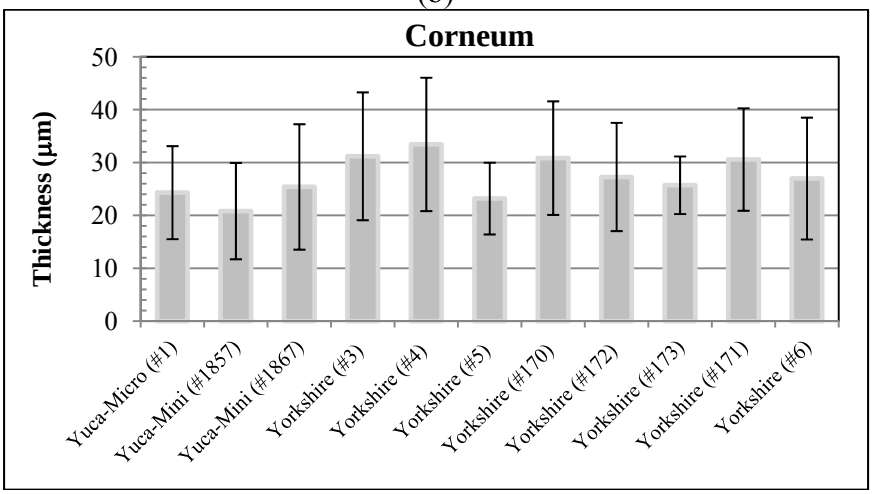


(c)

Figure 4. Averaged skin layer thickness by subject number in the (a) dermis, (b) epidermis and (c) stratum corneum. Error bars represent standard deviation. Each bar represents the average from 60 measurements

Skin thickness data were then grouped by breed and averaged respectively as shown in Figure 5. Sample sizes for the skin thickness breed comparisons were as follows: Micro pig - 1 subject for a total of 60 thickness measurements per layer. Mini pig - 2 subjects for a total of 120 thickness measurements per layer. Yorkshire - 8 subjects for a total of 480 thickness measurements per layer.

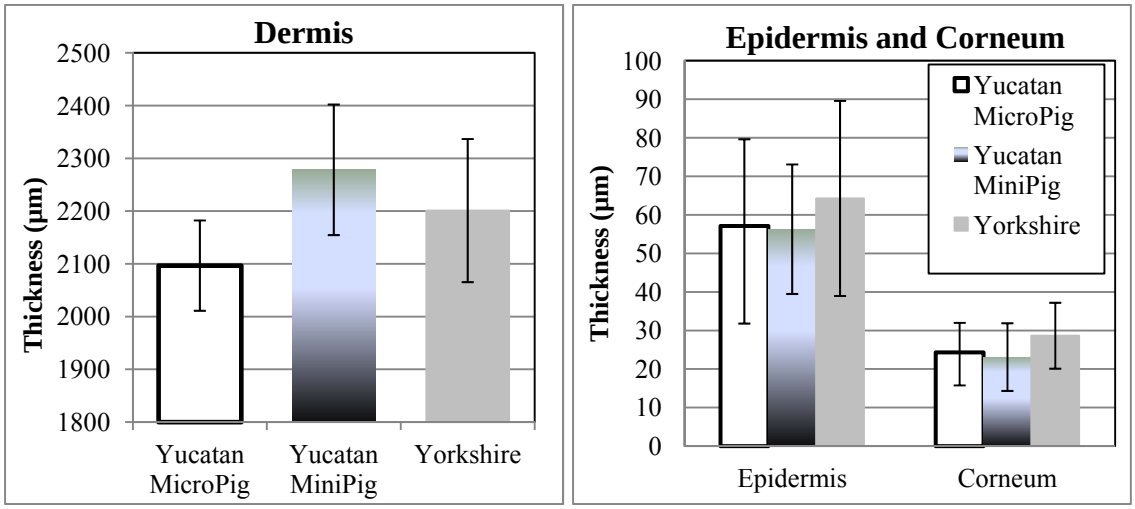


Figure 5. Skin thickness by breed in the (a) dermis and (b) epidermis and stratum corneum. Error bars are based on the standard deviation

Eggleston *et al*. (2000) reported the flank epidermis thickness between Yucatan Mini-pig (5 male and 1 female subject) and Yorkshire (6 female subjects). In this study, the thickness of the epidermis in the Yucatan Mini-pig (2 female subjects) and Yorkshire (8 male subjects) were measured. The reported epidermal skin thickness values from Eggleston *et al*. (2000) are contrasted against equivalent breeds used in this study in Figure 6.

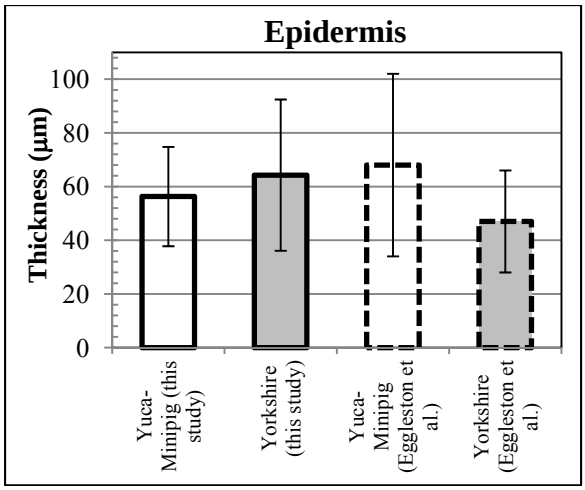


Figure 6. Comparison of epidermal skin thickness measurements, from the flank, determined in this study and those reported in Eggleston *et al*

Using the averaged skin-thickness data by breed from this study, an F-test was used (in Excel) to determine if the variances in the data between breeds were equal. If the p-value

was ≤ 0.05, then data between breeds were treated as having different variances. A student's t-test (two-tailed with equal or unequal variance based on the F-test results) was then run between each breed. Results from the F-test and t-tests between subjects are reported in Table 3.

Table 3. Statistical comparison (p-values) of skin thickness between breeds. Segments in gray indicate statistically different variances (F-test) or means (t-test)

p-Values from F-Test			
Dermis	Micro-Pig	Mini-Pig	Yorkshire
Micro-Pig			
Mini-Pig	0.2510		
Yorkshire	0.0000	0.0000	
Epidermis	Micro-Pig	Mini-Pig	Yorkshire
Micro-Pig			
Mini-Pig	0.0000		
Yorkshire	0.0000	0.0043	
Stratum Corneum	Micro-Pig	Mini-Pig	Yorkshire
Micro-Pig			
Mini-Pig	0.1918		
Yorkshire	0.0000	0.0000	

p-Values from t-Test			
Dermis	Micro-Pig	Mini-Pig	Yorkshire
Micro-Pig			
Mini-Pig	0.0000		
Yorkshire	0.0000	0.0047	
Epidermis	Micro-Pig	Mini-Pig	Yorkshire
Micro-Pig			
Mini-Pig	0.8083		
Yorkshire	0.0265	0.0001	
Stratum Corneum	Micro-Pig	Mini-Pig	Yorkshire
Micro-Pig			
Mini-Pig	0.4188		
Yorkshire	0.0006	0.0000	

3.2 Melanin Index

A total of six biopsies were acquired from each subject; 3 from the right and 3 from the left flank. For each individual biopsy, the melanin content index was calculated by dividing the area of melanin by the selected area of interest. The raw histological data by slide number, slide section (where each lettered slide section identifies an individual biopsy) and subject number are reported in Appendix 2. The results for the melanin index for each subject for the Micro-pig, Mini-pig, and Yorkshire breeds are shown in Figure 7,
Figure 8, and Figure 9 respectively. The x-axis denotes the histological slide section. Slide numbers are identified in the legends. Data are represented by the average and standard deviation of 3 index readings taken from each biopsy section. During biopsy collection, it was noted that Yorkshire subject #170 was noticeably highly pigmented.

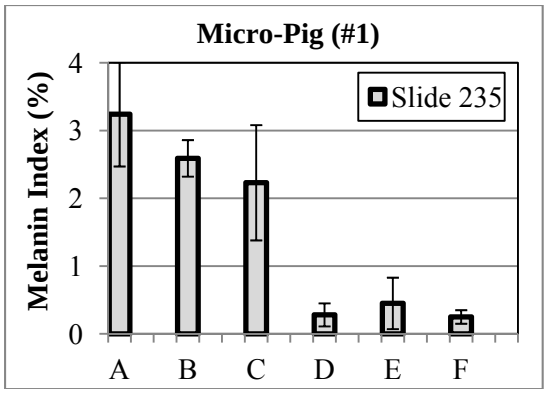


Figure 7. Melanin index values for the only Micro-pig subject

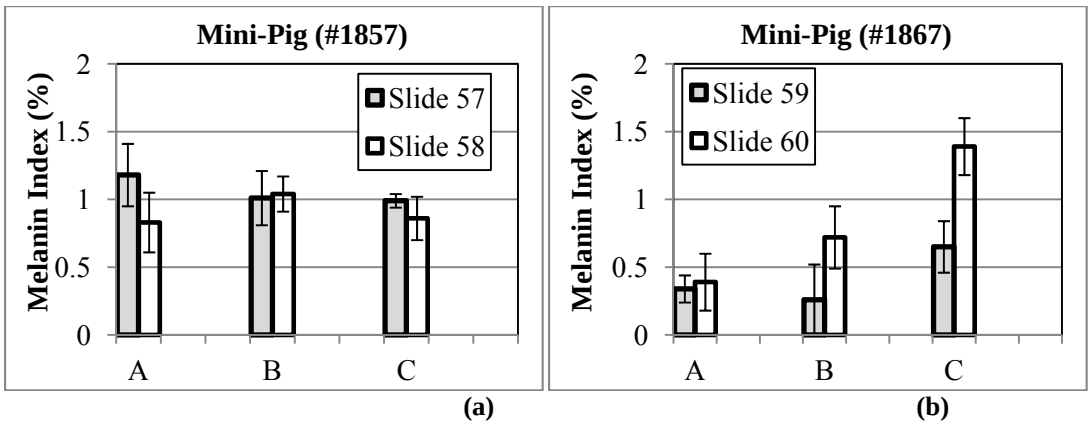


Figure 8. Melanin index values for the Mini-pig for subjects (a) 1857 and (b) 1867

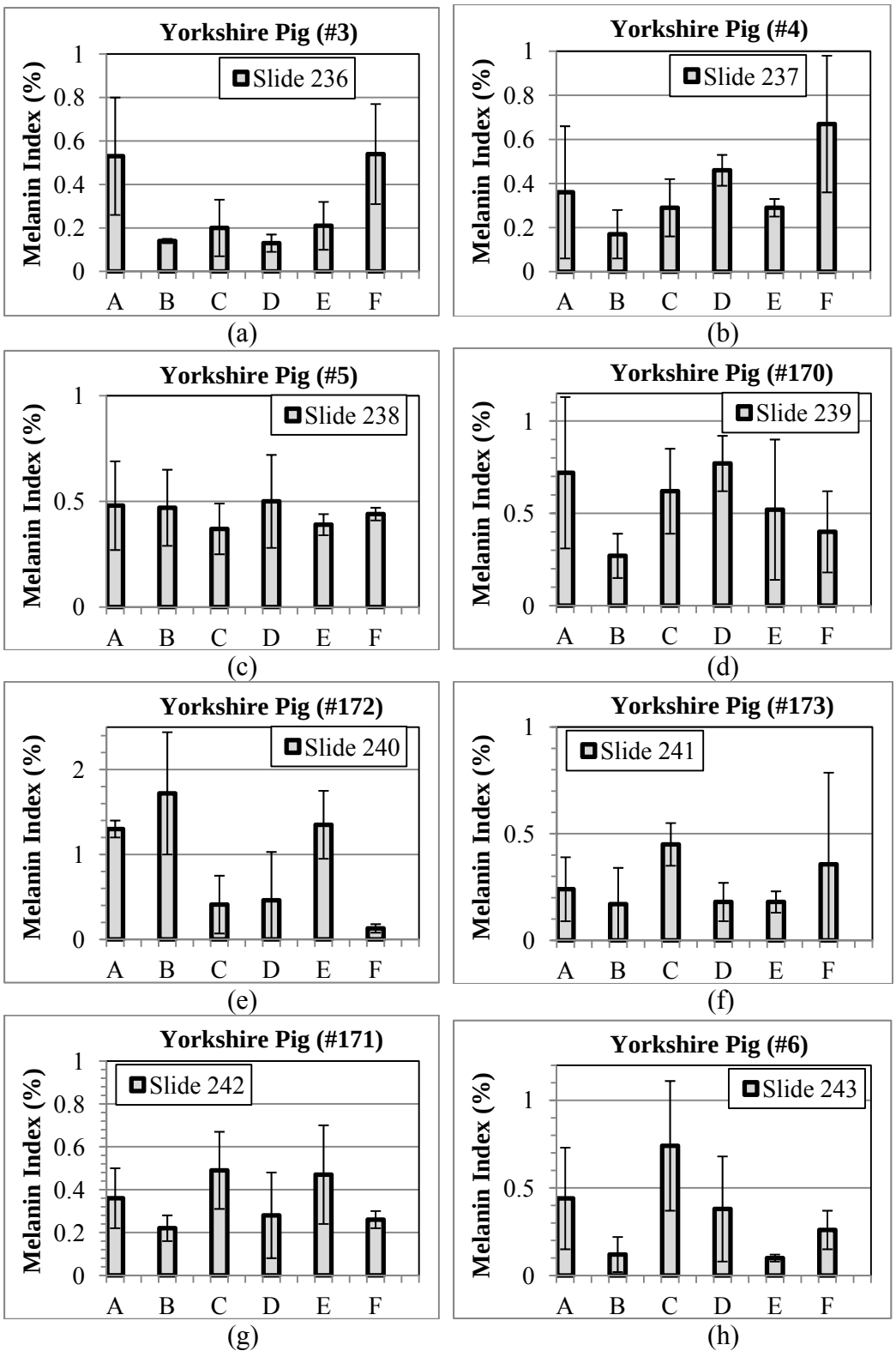


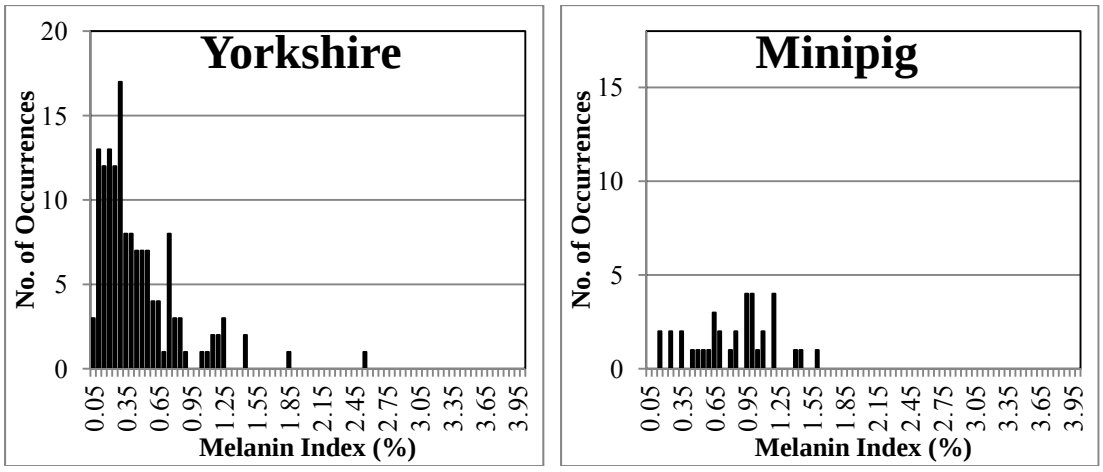
Figure 9. Melanin index values for the Yorkshire pig for subject numbers 3, 4, 5, 170, 172, 173, 171, & 6 in (a) thru (h) respectively

The maximum, minimum, average, and standard deviation for the melanin index values by breed are presented in Figure 4.

Table 4. Melanin index range (maximum and minimum), average and standard deviation for each breed. Sample sizes are given based upon number of subjects, biopsies acquired and reads

Breed	No. of Subjects	No. of biopsies (6*No. of subjects)	No. of reads (3*No. of biopsies)	Avg.	Standard Deviation	Max	Min
Yucatan Micro-pig	1	6	18	1.51	1.32	3.85	0.10
Yucatan Mini-pig	2	12	36	0.81	0.37	1.60	0.11
Yorkshire	8	48	144	0.44	0.37	2.54	0.02

The distributions of the melanin index data for each breed were examined in histograms by using a bin width of 0.05 % as shown in Figure 10.



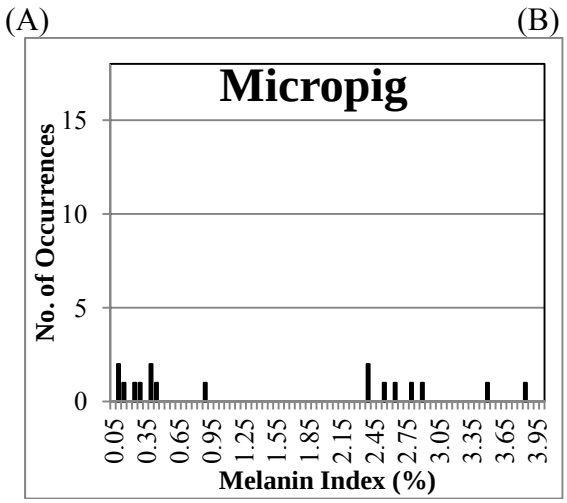


Figure 10. Histograms depicting the distribution of the melanin index reading for the (A) Yorkshire, (B) Mini-pig and (C) Micro-pig

4 DISCUSSION

4.1 Skin Thickness

Human skin stratum corneum, epidermis, and dermis measurements range from 8 to 20 μm (Holbrook & Odland, 1974), 50 to 100 μm (Gentilhomme & Neveux, 2004), and 200 to 500+ μm (Agache, 2004), respectively (not considering the skin on the palms or soles of the feet). The thickness of the epidermal layer varies greatly depending on the region of the body (i.e., the skin of the eyelid has a much thinner dermal layer, ~300 μm, compared to the thicker dermal layer found on the back, > 1000 μm). Skin thickness also varies with age. The three swine breeds in this study had stratum corneum, epidermis, and dermis measurements ranging from 15 to 35 μm (see Figure 5 b), 30 to 90 μm (see Figure 5 b) and 2000 to 2400 μm (see Figure 5 a), respectively. Collectively, all three swine breeds were found to have skin thickness ranges close to those reported for human skin.

While the histological structure of the skin appears similar across all three breeds (see Figure 3), general trends in the skin thickness measurements in this study differed from previously reported data. Eggleston *et al*. (2000) reported the flank epidermis thickness between Yucatan Mini-pig (5 male and 1 female subject) and Yorkshire (6 female subjects) as 68 +/- 34 and 47 +/- 19 μm, respectively (see Figure 6). In this study, the averaged thickness of the epidermis in the Yucatan Mini-pig (2 female subjects) and Yorkshire (8 male subjects) were found to be 56 +/- 18 and 64 +/- 28 μm, respectively. Clearly, differences in the Yorkshire pig data between these two studies could be influenced by the fact that Eggleston *et al*. used females and this study used males. The standard deviations were found to overlap between the Eggleston *et al*. data and this study (see Figure 6). This study did not use an equivalent subject number for sampling across breeds, though there were intra-breed variations between equivalent sample sizes in the Yorkshire group (see Appendix 3). Eggleston *et al*. did not report if they did or did not find statistically significant intra-breed variations in skin thickness measurements

between subjects. There did not appear to be any statistically significant differences between the 2 Yucatan Mini-pig subjects in epidermis thickness (see Appendix 3). Overall, epidermal thicknesses between the Yucatan Mini-pig and Yorkshire breeds were found to be statistically different (see Table 3), with the Yorkshire having the thicker epidermal and stratum corneum layers.

The Yucatan Micro-pig (unknown gender) was found to have skin thicknesses more similar to the Yucatan Mini-pig compared to the Yorkshire (see Table 3). The Micro-pig was found to have a significantly thinner epidermis, dermis, and stratum corneum compared to the Yorkshire breed. It is important to note that only 60 skin thickness measurements were acquired on 1 Micro-pig subject compared to averaging the 60 skin thickness measurements acquired on each of the Yorkshire subjects (8 Yorkshire subjects = 480 measurements). Thus, this study does not reflect a true sample size comparison, but does provide some expectations on the skin thicknesses in the new Micro-pig breed compared to the commonly utilized Mini-pig and Yorkshire breeds.

4.2 Melanin Index

A total of 18, 36, and 144 melanin indices were measured for each of the Micro-pig (1 of unknown gender), Mini-pig (2 females) and Yorkshire pig (8 males) breeds.
Figure 7,
Figure 8, and Figure 9, and Table 4 depict the large variation in melanin index values obtained. This is expected as melanin is non-uniformly distributed in the skin of a subject (i.e., random spots of pigment, etc.). It should be understood that melanogenesis, the molecular pathway responsible for producing melanin, is environmentally sensitive with effects capable of remaining localized to areas on the skin as opposed to the entire organ (Slominski *et al*., 2004). Such variability in melanogenesis further contributes to the non-uniformity of sampling for melanin content. Nonetheless, the amount of melanin would seem to follow a distribution, as depicted in Figure 10a, when the sample size is large. When the sample size is small, as in Figure 10 b & c (for the Mini- and Micro-pigs respectively), a distribution cannot be determined. Based on the distributions of the melanin index values in Figure 10, it appears the Yorkshire is most likely to have an average melanin index around 0.37%, with the Mini- and Micro-pigs trending towards melanin index values greater than that of the Yorkshire pig. The sample sizes for the Mini- and Micro-pigs were insufficient to assess the complete melanin index values between breeds.

5 CONCLUSION

Eggleston *et al*. (2000) previously reported that the thickness and pigmentation of skin from the Yucatan Mini-pig more closely resembled the skin of a human compared to the Yorkshire breed. Based on the limited sample size from this study, the Yucatan Micro-pig appears to be more comparable in skin thickness to the Yucatan Mini-pig when compared to the Yorkshire breed. Furthermore, the melanin index from this study indicates that the Yucatan Micro-pig may be more pigmented than both the Yucatan Mini-pig and Yorkshire breeds; however data were insufficient to determine the melanin index distribution for either of the Yucatan breeds. It appears the Yucatan Micro-pig could be used as a substitution for the Yucatan Mini-pig for skin experiments involving non-ionizing optical radiation.

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Appendix 1: Automated Melanin Index

Historically, melanin concentration has been done by laying a grid on a slide and counting the number of grid squares that contained melanin and comparing to the total number of squares, generating a ratio. Another technique included counting the number of cells containing melanin. Both techniques are acceptable, but have limitations. Using a grid either ignores the concentration of the melanin in a particular grid square, or assumes that the concentration will average out with large numbers of counts. Cell counting ignores the melanin concentration within each cell. Both of these limitations can be overcome by using an image processing technique that thresholds the image, and, after drawing a region of interest (ROI), determine area of image above the threshold in the ROI, and compares that to the total area of the ROI. All three techniques (grid, cell counting, and image processing) are sensitive to the staining method.

An example of the image-processing technique is provided. The slide was prepared as listed in Methods, and a Fontana-Masson stain used. All requirements for slide preparation are the same as for other methods. The specific software used, Image-Pro Plus Version 7.0 from Media Cybernetics, Inc., does not require that the image be captured before any analysis, and so can be accomplished without saving the image. Figure A1 is a representative image before any image processing.

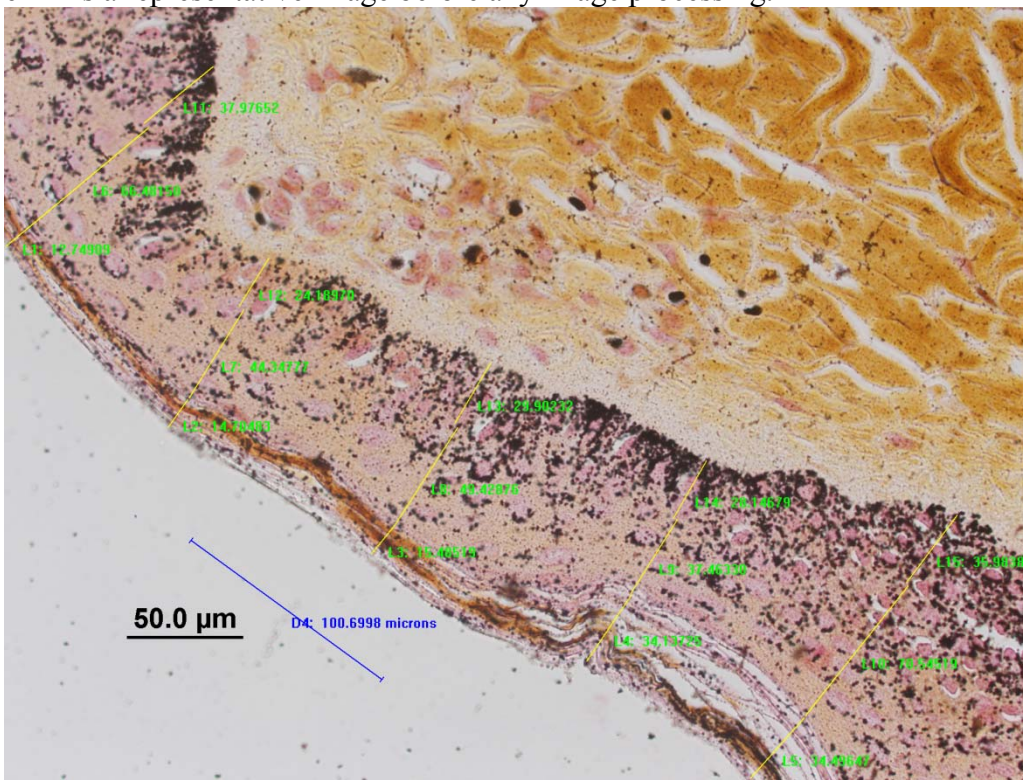

Figure A 1 Image to be processed for melanin concentration

Next, a threshold is applied, as shown in Figure A2, making areas at or above threshold appear red.

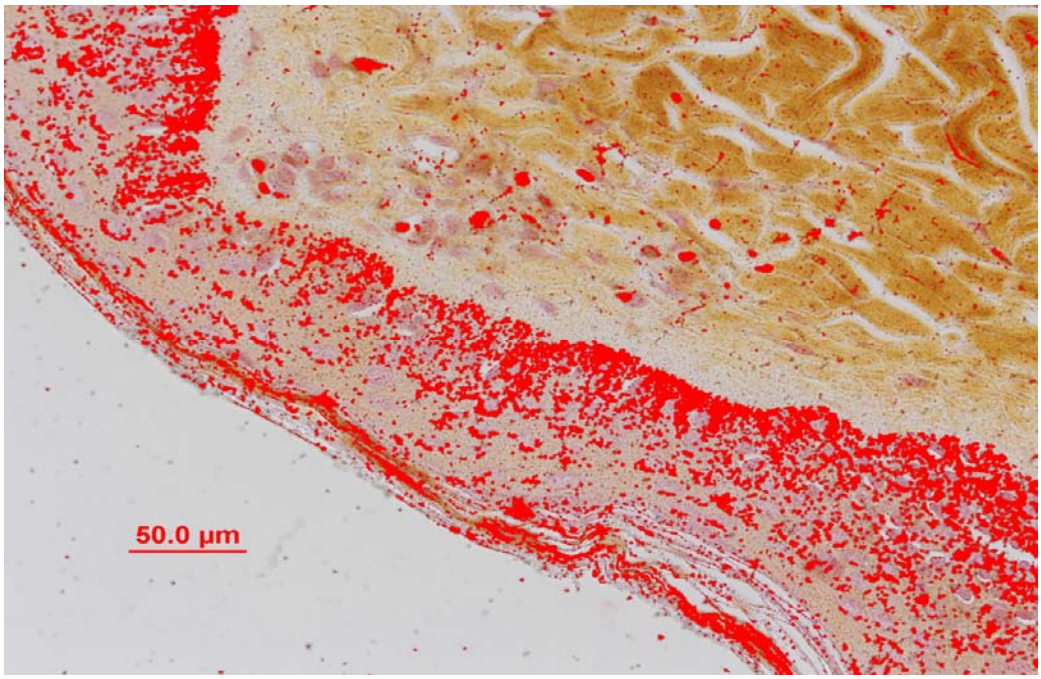

Figure A 2 Threshold applied

Now, a ROI is selected, and outlined in the software. For our purposes, only the epidermis was selected. Figure A3 demonstrates the ROI as it is drawn in red. The software then numbers the thresholded areas, and can be read on an expanded image.

The software calculates the area of each selected area, and generates the statistics shown in Table A1 for each ROI. The software also calculates the total area of the ROI. For our purposes, the sum of the melanin values was compared to the total area of the ROI to generate the Melanin Index.

Table A 1 Statistics from ROI

Statistics	Area
Minimum	0.45657098
Object number	32
Maximum	602.26276
Object number	69
Range	601.80621
Mean	7.9545345
Std. Dev.	35.720493
Sum	3770.5913
Samples	474

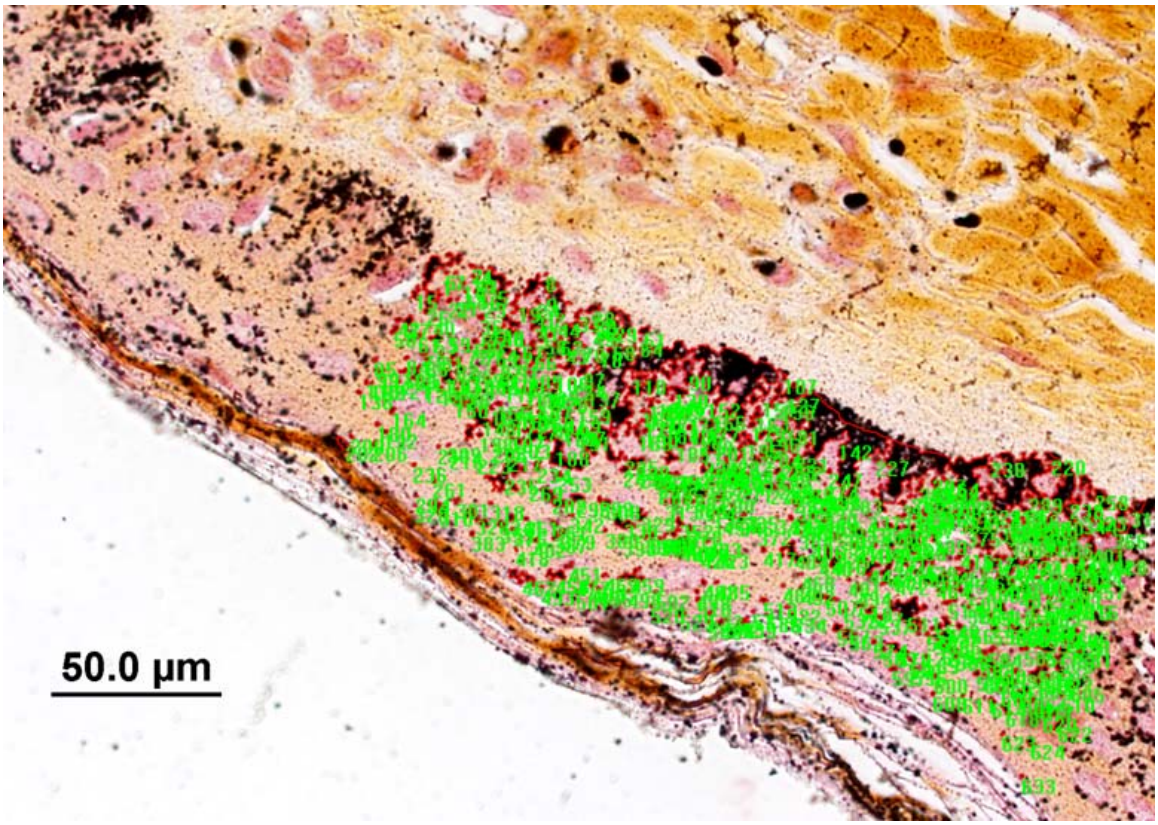

Figure A 3 ROI and numbered, threshold areas

The software generates an image of each of the threshold areas, sorted by size, and is shown in Figure A 4. Note the large variety of morphologies in the larger aggregations of melanin.

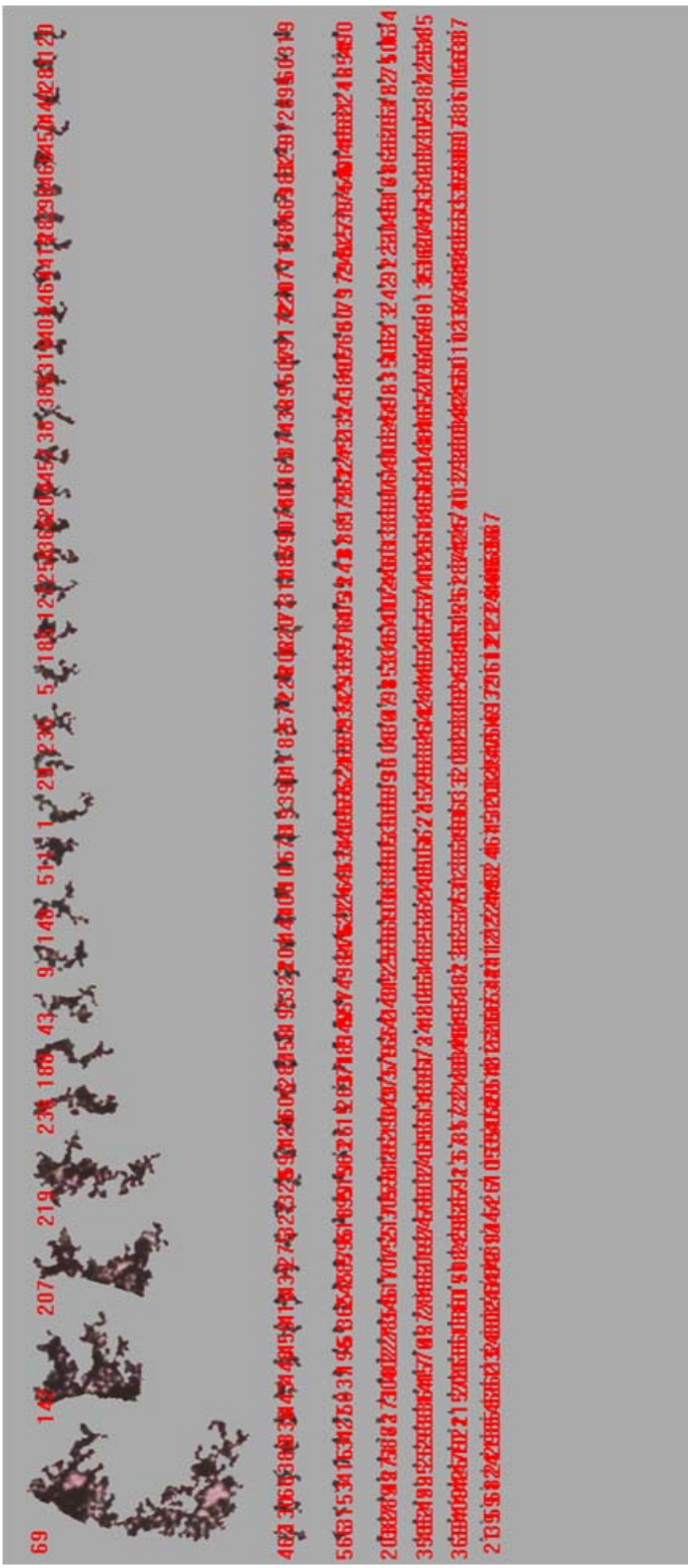

Figure A 4 Morphology of individual melanin regions from example image. Notice the size differences

Appendix 2: Histology

Histological results from each slide and corresponding subject number.

Skin Thickness Measurements for the Micro-Pig:

RHDV	Slide #	Slide Section	Line	Swine	Subject #	Sample date	Dermis thickness (µm)	Epidermis thickness (µm)	Corneum thickness (µm)
N09	235	A	1	Yuc-Micro	1	5-Jun-09	1988.950	98.832	29.880
			2				2038.674	88.968	38.002
			3				2160.221	35.938	16.575
			4				2154.696	39.286	31.162
			5				2099.448	45.580	38.674
			6				2060.773	72.930	43.896
			7				2038.674	45.307	50.655
			8				2077.348	91.160	30.387
			9				2044.199	86.445	21.839
			10				2060.773	53.885	29.038
N09	235	B	1	Yuc-Micro	1	5-Jun-09	1662.983	35.645	19.631
			2				1944.751	45.433	19.188
			3				1850.829	48.989	21.442
			4				1878.453	35.858	23.643
			5				1834.254	72.891	25.951
			6				1795.580	86.742	20.902
			7				1834.254	41.436	42.840
			8				1950.276	39.864	27.796
			9				1972.376	76.467	29.006
			10				2027.624	128.653	22.485
N09	235	C	1	Yuc-Micro	1	5-Jun-09	2016.575	109.674	18.167
			2				2016.575	69.226	24.900
			3				2055.249	35.645	12.734
			4				2033.149	92.139	19.776
			5				2027.624	59.392	34.558
			6				1966.851	60.648	18.787
			7				1917.127	49.724	18.009
			8				1955.801	43.678	28.474
			9				2088.398	48.343	27.624
			10				2220.994	48.500	45.914

RHDV	Slide #	Slide Section	Line	Swine	Subject #	Sample date	Dermis thickness (µm)	Epidermis thickness (µm)	Corneum thickness (µm)
N09	235	D	1	Yuc-Micro	1	5-Jun-09	2182.321	39.286	22.485
			2				2143.646	51.105	22.099
			3				2127.072	37.318	26.532
			4				2215.470	86.929	26.207
			5				2292.818	43.173	20.764
			6				2265.193	38.895	18.167
			7				2182.321	37.700	13.030
			8				2071.823	34.114	24.708
			9				2082.873	41.436	15.627
			10				2270.718	40.623	18.167
N09	235	E	1	Yuc-Micro	1	5-Jun-09	2243.094	35.214	7.438
			2				2298.343	38.302	12.354
			3				2292.818	95.414	15.442
			4				2254.144	35.938	22.271
			5				2270.718	61.662	20.111
			6				2331.492	58.077	16.689
			7				2397.790	88.268	17.688
			8				2342.542	53.369	19.188
			9				2403.315	55.969	30.668
			10				2331.492	37.573	27.967
N09	235	F	1	Yuc-Micro	1	5-Jun-09	2187.845	71.102	26.207
			2				2160.221	26.388	31.798
			3				2248.619	33.264	33.407
			4				1895.028	40.269	12.354
			5				1955.801	95.304	23.481
			6				2171.271	48.343	16.632
			7				2303.867	38.002	28.841
			8				1950.276	45.747	25.356
			9				2016.575	63.371	19.776
			10				2138.122	66.814	11.136

Skin Thickness Measurements for the Mini-Pig:

RHDV	Slide #	Slide Section	Line	Swine	Subject #	Sample date	Dermis thickness (µm)	Epidermis thickness (µm)	Corneum thickness (µm)

N10	57	A	1	Yuc-Mini	1857	25-Aug-09	2378.45	51.40	16.86
			2				2276.24	83.29	67.68
			3				2256.91	84.36	22.10
			4				2513.81	59.41	16.63
			5				2491.71	76.18	20.39
			6				2386.74	41.44	17.96
			7				2408.84	43.87	22.27
			8				2337.02	92.80	19.34
			9				2372.93	52.50	16.11
			10				2309.39	54.27	22.86
N10	57	B	1	Yuc-Mini	1857	25-Aug-09	1661.00	37.22	11.66
			2				1631.00	69.00	17.00
			3				1564.00	44.05	14.00
			4				1567.00	43.86	27.51
			5				1579.00	60.02	14.14
			6				1596.00	35.11	17.72
			7				1921.00	33.42	14.87
			8				1988.00	31.24	17.03
			9				2041.00	37.16	13.60
			10				1944.00	58.69	11.40
N10	57	C	1	Yuc-Mini	1857	25-Aug-09	2629.85	50.07	34.31
			2				2802.49	66.76	24.12
			3				2831.51	51.12	19.34
			4				2607.74	66.66	53.14
			5				2736.19	69.94	34.97
			6				2491.71	55.32	19.39
			7				2352.21	66.20	14.42
			8				2248.62	44.22	25.80
			9				2354.97	54.43	17.58
			10				2464.09	53.14	19.34

RHDV	Slide #	Slide Section	Line	Swine	Subject #	Sample date	Dermis thickness (µm)	Epidermis thickness (µm)	Corneum thickness (µm)
N10	58	A	1	Yuc-Mini	1857	25-Aug-09	2332.87	72.94	17.96
			2				2407.46	78.74	24.90
			3				2412.98	43.37	26.39
			4				2403.31	68.37	26.28

			5				2349.45	74.64	22.10
			6				2364.64	60.79	16.57
			7				2448.90	60.30	14.88
			8				2447.51	40.62	19.78
			9				1994.48	30.17	24.48
			10				2190.61	58.03	20.72
N10	58	B	1	Yuc-Mini	1857	25-Aug-09	2167.13	83.62	32.95
			2				2104.97	42.28	24.12
			3				2102.21	53.30	24.59
			4				2292.82	73.26	15.26
			5				2223.76	40.06	17.96
			6				2145.03	82.87	16.63
			7				2077.35	91.16	15.19
			8				2071.83	73.22	18.79
			9				1947.51	68.23	20.11
			10				2066.31	55.01	19.78
N10	58	C	1	Yuc-Mini	1857	25-Aug-09	2254.14	43.90	14.42
			2				2254.14	48.81	13.81
			3				2342.54	49.59	16.57
			4				2313.54	55.28	15.75
			5				2254.14	95.14	19.34
			6				2082.99	33.97	19.19
			7				2255.52	53.30	15.75
			8				2360.50	33.97	14.88
			9				2341.16	32.22	13.81
			10				2375.69	41.21	22.48

RHDV	Slide #	Slide Section	Line	Swine	Subject #	Sample date	Dermis thickness (µm)	Epidermis thickness (µm)	Corneum thickness (µm)
N10	59	A	1	Yuc-Mini	1867	25-Aug-09	2529.01	33.41	16.57
			2				2337.02	37.52	20.76
			3				2237.57	60.79	23.48
			4				2259.67	67.69	23.48
			5				2472.39	55.68	22.27
			6				2143.65	54.99	18.01
			7				2198.90	37.19	24.90

			8				2204.42	63.55	52.49
			9				2182.32	85.20	24.12
			10				2165.75	53.74	29.14
N10	59	B	1	Yuc-Mini	1867	25-Aug-09	2251.38	60.54	13.03
			2				2198.90	40.62	14.42
			3				2093.92	48.42	18.43
			4				2127.07	69.41	14.22
			5				2099.45	34.11	16.86
			6				2016.58	62.78	20.53
			7				2016.57	50.07	19.78
			8				2077.35	33.61	16.63
			9				2122.93	39.07	13.03
			10				2066.30	46.90	20.11
N10	59	C	1	Yuc-Mini	1867	25-Aug-09	2372.93	34.53	19.39
			2				2441.99	62.46	29.14
			3				2292.82	74.64	22.48
			4				2211.33	44.93	15.63
			5				2022.10	44.97	17.58
			6				1940.61	64.93	16.86
			7				1911.60	50.67	22.31
			8				2027.62	41.55	73.22
			9				2067.68	35.94	18.43
			10				2067.68	44.54	18.58

RHDV	Slide #	Slide Section	Line	Swine	Subject #	Sample date	Dermis thickness (µm)	Epidermis thickness (µm)	Corneum thickness (µm)
N10	60	A	1	Yuc-Mini	1867	25-Aug-09	2270.72	56.90	30.42
			2				2292.82	38.67	17.96
			3				2298.34	80.30	27.62
			4				2337.02	52.50	24.86
			5				2279.01	68.02	31.89
			6				2291.44	59.01	27.21
			7				2338.40	33.21	27.52
			8				2335.64	86.35	22.10
			9				2348.07	102.81	25.01
			10				2392.27	121.30	29.14

N10	60	B	1	Yuc-Mini	1867	25-Aug-09	2667.13	42.08	19.78
			2				2694.75	38.90	26.24
			3				2650.55	52.94	58.08
			4				2613.26	57.68	24.90
			5				2649.18	35.51	30.88
			6				2624.31	39.58	25.36
			7				2559.39	86.30	20.53
			8				2723.78	52.60	22.31
			9				2511.05	36.02	22.27
			10				2265.19	43.02	16.80
N10	60	C	1	Yuc-Mini	1867	25-Aug-09	2632.60	63.55	17.96
			2				2567.68	88.41	27.66
			3				2548.34	87.80	19.92
			4				2567.68	57.71	19.24
			5				2647.79	44.29	27.66
			6				2475.14	48.42	58.01
			7				2353.59	40.65	60.41
			8				2342.54	47.47	30.67
			9				2447.51	110.81	27.66
			10				2389.50	41.53	27.52

Skin Thickness Measurements for the Yorkshire-Pig:

RHDV	Slide #	Slide Section	Line	Swine	Subject #	Sample date	Dermis thickness (μm)	Epidermis thickness (μm)	Corneum thickness (μm)
N09	236	A	1	Yorkshire	3	13-May-09	2453.039	62.537	31.039
			2				2447.514	45.914	25.205
			3				2370.166	60.648	31.798
			4				2392.291	38.895	27.521
			5				2408.840	50.410	25.468
			6				2441.989	40.269	30.885
			7				2430.939	67.680	26.243
			8				2408.840	92.170	31.557
			9				2436.464	63.776	27.624
			10				2475.138	51.180	26.243
N09	236	B	1	Yorkshire	3	13-May-09	2795.580	40.997	28.340
			2				2839.779	43.896	26.061
			3				2861.878	30.418	19.337

			4				2773.503	50.049	29.365
			5				2596.685	35.376	53.938
			6				2508.287	48.657	41.459
			7				2524.862	35.511	59.505
			8				2701.657	58.633	47.245
			9				2552.486	39.576	29.559
			10				2397.790	42.008	26.532
N09	236	C	1	Yorkshire	3	13-May-09	2812.155	77.348	21.129
			2				2790.055	92.912	41.803
			3				2856.354	59.392	22.099
			4				2784.530	42.840	23.481
			5				2812.155	60.836	20.764
			6				2812.155	67.806	27.659
			7				2834.254	58.421	23.643
			8				2784.530	47.687	27.521
			9				2756.906	63.371	23.602
			10				2729.282	73.943	26.388

RHDV	Slide #	Slide Section	Line	Swine	Subject #	Sample date	Dermis thickness (μm)	Epidermis thickness (μm)	Corneum thickness (μm)
N09	236	D	1	Yorkshire	3	13-May-09	2187.845	69.075	28.474
			2				2204.420	85.646	24.592
			3				2071.831	36.255	29.559
			4				2066.298	38.674	22.143
			5				2121.554	96.685	24.862
			6				1972.376	44.048	22.485
			7				1828.738	35.912	24.708
			8				1977.901	63.551	30.418
			9				1906.077	136.034	23.153
			10				2160.221	45.914	34.530
N09	236	E	1	Yorkshire	3	13-May-09	2055.249	37.318	28.474
			2				2132.597	66.413	19.238
			3				2248.619	44.628	19.238
			4				2508.287	41.436	17.956
			5				2408.840	129.834	66.298
			6				2491.713	158.232	40.623

			7				2618.784	139.530	22.314
			8				2867.403	60.679	29.527
			9				1928.177	44.628	24.122
			10				1950.276	99.572	30.292
N09	236	F	1	Yorkshire	3	13-May-09	3049.724	35.912	19.776
			2				3049.729	43.896	25.951
			3				3033.149	61.165	26.568
			4				3005.525	97.686	32.598
			5				2983.425	62.216	45.664
			6				2911.602	40.810	82.608
			7				2906.077	106.926	43.612
			8				2928.177	98.095	49.915
			9				3022.099	60.332	41.066
			10				2895.028	47.144	37.165

RHDV	Slide #	Slide Section	Line	Swine	Subject #	Sample date	Dermis thickness (µm)	Epidermis thickness (µm)	Corneum thickness (µm)
N09	237	A	1	Yorkshire	4	13-May-09	1801.105	117.728	20.393
			2				1784.530	79.836	27.967
			3				1972.376	66.024	51.124
			4				1972.376	66.327	36.778
			5				1917.127	197.108	33.264
			6				2204.482	57.565	36.491
			7				2281.768	47.204	42.257
			8				2309.392	65.020	26.243
			9				2254.144	77.311	24.122
			10				1839.779	55.524	45.768
N09	237	B	1	Yorkshire	4	13-May-09	1121.547	44.586	27.967
			2				1165.746	56.949	28.474
			3				1320.442	65.619	24.122
			4				1441.989	45.244	14.420
			5				1546.961	53.867	24.900
			6				1541.437	81.034	32.126
			7				1668.508	59.776	51.773
			8				1795.580	97.481	31.039

			9				1624.309	34.169	25.803
			10				1701.657	45.559	20.533
N09	237	C	1	Yorkshire	4	13-May-09	1447.514	58.011	34.778
			2				1480.663	45.914	20.533
			3				1569.061	46.921	32.245
			4				1679.558	61.351	37.573
			5				1801.105	43.678	26.818
			6				1856.354	94.671	27.521
			7				2276.243	45.914	21.839
			8				2215.470	51.273	27.242
			9				1734.807	53.136	40.150
			10				1723.757	38.699	23.521

RHDV	Slide #	Slide Section	Line	Swine	Subject #	Sample date	Dermis thickness (µm)	Epidermis thickness (µm)	Corneum thickness (µm)
N09	237	D	1	Yorkshire	4	13-May-09	2878.453	58.093	29.267
			2				2872.928	29.300	20.111
			3				2861.878	37.522	22.780
			4				2662.983	121.547	17.085
			5				2928.177	91.160	30.387
			6				3077.348	66.356	26.279
			7				2928.177	55.266	23.843
			8				2939.227	34.778	23.643
			9				2955.801	50.277	33.861
			10				2801.105	102.266	33.635
N09	237	E	1	Yorkshire	4	13-May-09	1883.978	41.206	24.900
			2				1729.282	33.635	73.827
			3				1740.332	162.356	26.818
			4				1861.878	74.701	34.530
			5				1856.354	44.969	27.762
			6				1806.630	45.768	29.038
			7				1856.354	44.393	25.205
			8				1756.906	92.459	60.679
			9				1817.680	61.847	46.430
			10				1817.680	65.093	59.392

N09	237	F	1	Yorkshire	4	13-May-09	1701.657	60.773	45.601
			2				1745.856	52.595	35.912
			3				1696.133	82.063	56.088
			4				1596.685	68.185	67.680
			5				1740.332	37.700	36.491
			6				1569.061	144.401	22.863
			7				1513.812	87.748	56.088
			8				1464.088	54.780	31.798
			9				1370.166	74.866	33.436
			10				1530.387	35.912	32.831

RHDV	Slide #	Slide Section	Line	Swine	Subject #	Sample date	Dermis thickness (µm)	Epidermis thickness (µm)	Corneum thickness (µm)
N09	238	A	1	Yorkshire	5	13-May-09	1961.326	31.039	25.803
			2				2060.773	45.747	29.038
			3				2127.072	118.600	27.967
			4				2088.398	35.858	17.085
			5				2154.696	61.847	23.153
			6				2204.420	45.914	16.689
			7				2226.519	41.459	22.485
			8				2182.321	37.062	22.863
			9				2303.867	85.736	29.006
			10				2209.945	103.591	23.481
N09	238	B	1	Yorkshire	5	13-May-09	2132.597	106.363	24.900
			2				2116.022	46.327	23.153
			3				2226.519	74.790	19.776
			4				2276.243	49.300	27.624
			5				2198.895	58.681	20.393
			6				2171.271	67.680	29.006
			7				2359.116	53.867	35.938
			8				2364.641	38.377	21.083
			9				2198.895	81.246	20.111
			10				2187.845	78.729	22.099
N09	238	C	1	Yorkshire	5	13-May-09	1917.127	44.586	20.533
			2				1933.702	69.515	23.481
			3				1950.276	88.247	25.393

			4				2077.348	92.552	20.718
			5				2000.000	92.139	23.153
			6				2077.348	78.133	33.178
			7				2154.696	48.343	17.956
			8				2149.171	40.269	27.967
			9				2193.370	90.867	18.531
			10				2204.420	58.011	30.387

RHDV	Slide #	Slide Section	Line	Swine	Subject #	Sample date	Dermis thickness (µm)	Epidermis thickness (µm)	Corneum thickness (µm)
N09	238	D	1	Yorkshire	5	13-May-09	1944.751	49.589	23.843
			2				2000.000	70.388	22.780
			3				1994.475	67.000	16.803
			4				1939.227	32.510	23.521
			5				1850.829	29.267	31.888
			6				1922.652	53.351	20.111
			7				1917.127	37.190	24.122
			8				1861.878	92.912	22.780
			9				1977.901	31.798	16.575
			10				1922.652	55.867	17.471
N09	238	E	1	Yorkshire	5	13-May-09	2121.547	48.046	30.292
			2				1972.376	67.454	47.204
			3				1889.503	51.105	39.286
			4				1950.276	44.479	28.741
			5				2104.972	53.637	21.083
			6				2121.547	116.915	38.526
			7				2182.321	102.210	12.507
			8				2320.442	73.204	13.812
			9				2331.492	49.724	16.575
			10				2209.945	45.559	18.428
N09	238	F	1	Yorkshire	5	13-May-09	1535.912	73.568	24.592
			2				1629.834	52.486	26.279
			3				1591.160	49.300	15.442
			4				1497.238	64.327	19.776
			5				1475.138	57.300	18.428

			6				1569.061	66.714	14.683
			7				1983.425	91.171	15.193
			8				2099.448	48.362	14.086
			9				2022.099	63.386	15.748
			10				1939.227	59.054	17.471

RHDV	Slide #	Slide Section	Line	Swine	Subject #	Sample date	Dermis thickness (μm)	Epidermis thickness (μm)	Corneum thickness (μm)
N09	239	A	1	Yorkshire	170	7-May-09	2110.497	79.261	30.885
			2				2093.923	80.834	26.532
			3				1817.680	39.768	32.510
			4				2066.298	74.866	38.699
			5				2182.321	26.818	26.818
			6				2243.094	62.583	30.324
			7				2176.802	47.245	18.009
			8				2044.199	73.217	27.659
			9				2160.221	49.512	26.207
			10				2077.348	58.077	31.888
N09	239	B	1	Yorkshire	170	7-May-09	1690.608	50.787	41.758
			2				1723.757	55.404	31.798
			3				1734.807	106.273	22.780
			4				1696.133	154.159	57.681
			5				1701.657	33.149	30.387
			6				1745.856	39.067	55.679
			7				1745.856	34.169	40.222
			8				2176.796	30.292	30.668
			9				2248.619	109.256	35.912
			10				2281.768	44.285	40.150
N09	239	C	1	Yorkshire	170	7-May-09	2359.116	107.814	20.902
			2				2497.244	36.255	20.533
			3				2165.746	70.780	48.343
			4				2193.370	39.840	33.635
			5				2104.972	49.454	22.485
			6				2088.398	30.418	33.436
			7				2226.519	31.527	18.787

			8				2248.619	74.598	53.726
			9				2348.066	67.454	29.527
			10				2359.116	52.776	26.243

RHDV	Slide #	Slide Section	Line	Swine	Subject #	Sample date	Dermis thickness (µm)	Epidermis thickness (µm)	Corneum thickness (µm)
N09	239	D	1	Yorkshire	170	7-May-09	2154.696	92.634	29.137
			2				2530.387	129.864	31.162
			3				2541.437	128.535	22.485
			4				2596.685	79.705	18.531
			5				2491.713	119.433	24.436
			6				2502.762	59.968	23.843
			7				2546.961	34.530	25.205
			8				2436.464	83.755	45.914
			9				2215.470	56.781	27.762
			10				1950.276	55.969	34.530
N09	239	E	1	Yorkshire	170	7-May-09	2182.321	26.676	22.863
			2				2220.994	39.091	24.161
			3				2248.619	93.923	23.643
			4				2314.924	58.011	19.337
			5				2370.166	67.680	27.624
			6				2408.840	52.486	29.006
			7				2359.116	114.899	33.635
			8				2303.867	103.517	34.530
			9				2287.293	51.569	44.990
			10				2381.216	75.854	65.633
N09	239	F	1	Yorkshire	170	7-May-09	2204.420	52.595	34.114
			2				2215.470	36.255	26.388
			3				2116.022	58.274	27.659
			4				2132.597	62.155	20.718
			5				1955.809	39.286	16.575
			6				1922.652	40.269	19.238
			7				1928.177	60.332	22.863
			8				1856.354	80.372	21.839
			9				1850.829	50.049	50.201

			10				1823.204	97.314	17.956

RHDV	Slide #	Slide Section	Line	Swine	Subject #	Sample date	Dermis thickness (µm)	Epidermis thickness (µm)	Corneum thickness (µm)
N09	240	A	1	Yorkshire	172	7-May-09	2309.392	57.565	23.153
			2				2375.691	46.900	27.796
			3				2392.265	32.831	25.468
			4				2353.591	48.046	20.533
			5				2359.116	112.227	21.129
			6				2298.343	47.245	60.538
			7				2276.243	30.512	23.643
			8				2099.448	39.552	30.885
			9				2215.470	69.460	31.888
			10				2071.823	63.596	24.862
N09	240	B	1	Yorkshire	172	7-May-09	2022.099	66.097	25.205
			2				2044.199	50.030	34.641
			3				2154.696	37.190	22.780
			4				2204.420	37.573	37.190
			5				2337.017	50.580	25.692
			6				2441.989	53.351	27.207
			7				2403.315	92.541	13.812
			8				2403.315	46.327	21.129
			9				1657.459	42.257	22.099
			10				1878.453	42.550	29.300
N09	240	C	1	Yorkshire	172	7-May-09	2486.188	37.927	25.015
			2				2270.718	56.377	27.624
			3				2276.243	53.885	26.818
			4				2281.768	44.220	29.300
			5				2232.044	58.224	27.796
			6				2127.072	30.387	25.468
			7				2016.575	53.136	24.122
			8				2116.022	37.725	24.122
			9				2071.823	40.222	22.485
			10				2143.646	42.008	29.527

RHDV	Slide #	Slide Section	Line	Swine	Subject #	Sample date	Dermis thickness (µm)	Epidermis thickness (µm)	Corneum thickness (µm)
N09	240	D	1	Yorkshire	172	7-May-09	1911.602	36.150	29.006
			2				1850.829	80.110	24.862
			3				1723.757	79.104	28.841
			4				1712.707	25.393	27.967
			5				1823.204	45.914	84.807
			6				2093.923	38.699	35.214
			7				2088.398	48.362	31.798
			8				2110.497	45.244	34.114
			9				2127.072	64.917	31.768
			10				1944.751	58.077	19.386
N09	240	E	1	Yorkshire	172	7-May-09	2414.365	59.968	27.624
			2				2397.790	30.885	19.776
			3				2314.917	45.096	18.582
			4				2215.470	70.021	32.831
			5				2226.519	68.185	18.428
			6				2198.895	95.793	23.643
			7				2093.923	52.595	25.951
			8				2011.050	46.921	20.533
			9				2077.355	71.783	14.420
			10				2000.000	29.880	26.532
N09	240	F	1	Yorkshire	172	7-May-09	2016.575	34.530	24.862
			2				1961.326	107.735	21.038
			3				1928.177	54.027	38.476
			4				1966.851	60.174	19.631
			5				2082.873	100.136	20.533
			6				2077.348	46.961	29.038
			7				2132.597	38.203	28.340
			8				2232.044	53.154	25.356
			9				2276.243	49.358	25.803
			10				2386.740	45.726	15.442

RHDV	Slide #	Slide Section	Line	Swine	Subject #	Sample date	Dermis thickness (µm)	Epidermis thickness (µm)	Corneum thickness (µm)
N09	241	A	1	Yorkshire	173	6-May-09	1790.055	31.798	26.388
			2				1872.928	56.377	36.465
			3				2044.199	35.403	27.521
			4				1839.779	144.190	26.207
			5				1806.630	55.524	26.818
			6				2171.271	36.491	18.787
			7				1977.908	84.976	30.885
			8				1944.751	92.139	25.015
			9				1939.227	70.807	21.839
			10				2093.923	73.204	27.659
N09	241	B	1	Yorkshire	173	6-May-09	1674.033	63.671	25.393
			2				1972.376	69.515	20.533
			3				2033.149	113.066	25.692
			4				2138.122	76.580	29.300
			5				2165.746	56.781	27.624
			6				2149.171	73.139	28.171
			7				2121.547	95.144	34.530
			8				2127.072	106.578	32.126
			9				2027.624	109.395	36.465
			10				1950.276	100.440	26.061
N09	241	C	1	Yorkshire	173	6-May-09	1872.928	38.895	22.485
			2				1773.481	44.069	24.161
			3				1900.552	62.170	31.768
			4				1972.376	63.386	29.300
			5				1917.135	75.564	29.656
			6				1795.580	68.143	29.267
			7				1795.580	84.435	24.475
			8				1762.431	34.613	19.920
			9				1712.707	48.105	31.497
			10				1640.884	55.679	26.388

RHDV	Slide #	Slide Section	Line	Swine	Subject #	Sample date	Dermis thickness (µm)	Epidermis thickness (µm)	Corneum thickness (µm)
N09	241	D	1	Yorkshire	173	6-May-09	2441.989	52.776	29.816
			2				2475.138	37.293	32.037
			3				2508.287	56.224	16.689
			4				2392.265	39.310	19.776
			5				2392.265	85.379	26.818
			6				2353.591	86.445	23.843
			7				2430.939	96.507	23.481
			8				2392.265	33.149	20.718
			9				2436.464	63.551	33.264
			10				2381.216	54.501	23.153
N09	241	E	1	Yorkshire	173	6-May-09	2292.818	54.168	22.863
			2				2337.017	36.150	20.718
			3				2265.193	43.371	17.956
			4				2381.216	43.612	15.442
			5				2414.365	39.552	37.190
			6				2364.641	55.969	26.207
			7				2314.917	42.550	25.468
			8				2265.193	51.403	29.006
			9				2502.762	40.079	22.143
			10				2486.188	20.718	21.619
N09	241	F	1	Yorkshire	173	6-May-09	2005.525	56.647	26.818
			2				2016.582	110.169	27.967
			3				2077.348	70.564	13.603
			4				2066.298	36.334	13.603
			5				2082.873	59.968	15.627
			6				2187.845	35.376	25.393
			7				2160.221	32.510	22.271
			8				2149.171	47.627	30.387
			9				2248.619	44.543	28.474
			10				2251.382	77.471	27.659

RHDV	Slide #	Slide Section	Line	Swine	Subject #	Sample date	Dermis thickness (µm)	Epidermis thickness (µm)	Corneum thickness (µm)
N09	242	A	1	Yorkshire	171	6-May-09	2410.221	79.932	25.205
			2				2455.801	34.778	18.009
			3				2408.840	85.613	24.862
			4				2388.122	68.339	31.497
			5				2359.116	73.529	41.459
			6				2359.116	39.768	27.796
			7				2370.166	58.616	29.559
			8				2338.398	43.612	37.190
			9				2220.994	35.214	28.441
			10				2292.818	35.160	23.440
N09	242	B	1	Yorkshire	171	6-May-09	2265.193	30.166	23.602
			2				2265.194	82.237	32.831
			3				2276.243	32.245	23.521
			4				2303.867	35.160	25.393
			5				2309.399	35.938	26.243
			6				2237.569	36.465	25.468
			7				2298.343	47.466	32.831
			8				2099.448	55.610	24.475
			9				2127.072	41.436	22.654
			10				2290.056	80.455	67.270
N09	242	C	1	Yorkshire	171	6-May-09	2558.011	58.093	37.573
			2				2426.796	97.246	33.407
			3				2469.613	27.624	19.337
			4				2364.641	41.940	34.641
			5				2408.840	47.204	27.796
			6				2419.889	112.558	32.510
			7				2414.422	44.069	33.436
			8				2364.641	43.018	21.575
			9				2447.514	35.832	19.188
			10				2425.414	107.814	26.388

RHDV	Slide #	Slide Section	Line	Swine	Subject #	Sample date	Dermis thickness (µm)	Epidermis thickness (µm)	Corneum thickness (µm)
N09	242	D	1	Yorkshire	171	6-May-09	2591.160	100.069	49.800
			2				2439.227	96.111	34.778
			3				2381.216	76.977	29.300
			4				2259.669	56.899	34.970
			5				2182.321	131.940	29.880
			6				2215.470	177.555	26.568
			7				2182.321	129.193	30.324
			8				2316.299	36.570	22.780
			9				2255.525	73.840	22.863
			10				2276.243	99.601	40.222
N09	242	E	1	Yorkshire	171	6-May-09	2472.376	104.023	43.896
			2				2538.674	54.693	17.580
			3				2447.514	97.246	40.055
			4				2475.138	59.920	37.700
			5				2635.359	85.947	23.440
			6				2674.033	89.875	22.271
			7				2533.150	104.699	21.083
			8				2515.194	32.744	24.161
			9				2465.470	40.269	25.468
			10				2729.282	55.969	34.169
N09	242	F	1	Yorkshire	171	6-May-09	2552.486	90.161	38.203
			2				2469.613	72.668	66.413
			3				2425.421	28.340	26.818
			4				2479.282	72.930	34.530
			5				2624.309	88.925	40.150
			6				2563.536	77.656	31.768
			7				2607.735	78.572	25.015
			8				2812.155	60.648	24.708
			9				2611.879	47.466	34.641
			10				2722.376	95.942	23.153

RHDV	Slide #	Slide Section	Line	Swine	Subject #	Sample date	Dermis thickness (µm)	Epidermis thickness (µm)	Corneum thickness (µm)
N09	243	A	1	Yorkshire	6	14-May-09	2639.503	48.520	23.843
			2				2501.383	48.362	27.624
			3				2447.514	118.503	32.126
			4				2563.536	66.413	39.768
			5				2726.520	44.393	27.762
			6				2497.238	93.963	33.379
			7				2378.453	37.190	19.776
			8				2298.343	39.552	14.086
			9				2100.829	102.926	38.402
			10				2027.624	99.448	27.659
N09	243	B	1	Yorkshire	6	14-May-09	1694.751	75.210	7.438
			2				2084.254	124.048	7.043
			3				2060.773	58.027	8.287
			4				1950.276	57.300	8.287
			5				1662.983	57.300	8.287
			6				1607.735	132.431	25.356
			7				1574.586	40.810	44.820
			8				1546.961	35.403	27.967
			9				1458.563	98.154	29.752
			10				1564.918	127.619	23.153
N09	243	C	1	Yorkshire	6	14-May-09	2433.702	55.969	31.039
			2				2440.608	100.715	21.038
			3				2515.194	81.069	25.015
			4				2477.901	128.460	32.598
			5				2464.088	62.537	43.896
			6				2441.989	65.283	36.018
			7				2513.813	109.221	36.570
			8				2523.481	63.671	22.143
			9				2408.840	46.594	36.855
			10				2736.188	106.997	38.526

RHDV	Slide #	Slide Section	Line	Swine	Subject #	Sample date	Dermis thickness (µm)	Epidermis thickness (µm)	Corneum thickness (µm)
N09	243	D	1	Yorkshire	6	14-May-09	2135.359	97.049	30.292
			2				2174.037	55.145	21.619
			3				2303.867	34.942	22.314
			4				2314.917	63.129	30.512
			5				2265.193	102.963	29.038
			6				2374.309	44.969	26.388
			7				2375.691	49.896	22.143
			8				2509.668	38.203	16.803
			9				2535.912	42.076	17.956
			10				2326.026	44.220	23.521
N09	243	E	1	Yorkshire	6	14-May-09	2060.773	45.601	22.099
			2				2000.000	119.073	23.643
			3				1939.227	53.351	53.316
			4				1708.563	29.006	31.798
			5				2059.392	30.418	66.370
			6				2049.731	61.305	56.882
			7				2051.105	58.763	39.768
			8				1895.028	161.342	31.497
			9				2040.056	81.539	22.271
			10				2541.437	93.373	27.933
N09	243	F	1	Yorkshire	6	14-May-09	2604.972	129.016	21.575
			2				2805.249	50.655	15.256
			3				2701.657	45.244	17.307
			4				2577.348	73.400	17.471
			5				2596.688	82.434	22.271
			6				2486.188	140.056	21.083
			7				2375.691	63.985	23.153
			8				2497.238	44.586	15.748
			9				2618.784	73.400	23.481
			10				2712.707	106.997	26.279

Melanin Content Measurements for the Micro-Pig:

RHDV	Slide #	Slide Section	Area	Swine	Subject #	Sample date	Melanin area, A_M (μm²)	Epidermis area, A_E (μm²)	Melanin content index = A_M/A_E	Melanin (% epidermal area)	Average melanin % for sample +/- standard deviation
N09	235	A	1	Micro-Pig	1	5-Jun-09	627.00	16287.10	0.0385	3.85	3.24 +/- 0.77 %
			2				391.41	16421.92	0.0238	2.38	
			3				507.70	14522.99	0.0350	3.50	
		B	1				526.62	19996.94	0.0263	2.63	2.59 +/- 0.27 %
			2				381.45	13777.33	0.0277	2.77	
			3				373.56	15709.38	0.0238	2.38	
		C	1				291.75	10107.43	0.0289	2.89	2.23 +/- 0.85 %
			2				329.37	13032.08	0.0253	2.53	
			3				192.01	15140.26	0.0127	1.27	
		D	1				59.76	13806.33	0.0043	0.43	0.28 +/- 0.17 %
			2				13.93	13540.28	0.0010	0.10	
			3				38.76	13309.28	0.0029	0.29	
		E	1				18.93	16076.75	0.0012	0.12	0.45 +/- 0.38 %
			2				55.03	14634.94	0.0038	0.38	
			3				125.66	14427.23	0.0087	0.87	
		F	1				25.29	14256.40	0.0018	0.18	0.25 +/- 0.10 %
			2				43.30	11914.38	0.0036	0.36	
			3				32.03	14688.94	0.0022	0.22	

Melanin Content Measurements for the Mini-Pig:

RHDV	Slide #	Slide Section	Area	Swine	Subject #	Sample date	Melanin area, A_M (μm²)	Epidermis area, A_E (μm²)	Melanin content index = A_M/A_E	Melanin (% epidermal area)	Average melanin % for sample +/- standard deviation

N10	57	A	1	Mini-Pig	1857	25-Aug-09	118.89	12600.02	0.0094	0.94	1.18 +/- 0.23 %
			2				221.63	15720.27	0.0141	1.41	
			3				205.71	17416.45	0.0118	1.18	
		B	1				136.78	17574.97	0.0078	0.78	1.01 +/- 0.20 %
			2				276.45	23628.58	0.0117	1.17	
			3				161.88	15130.11	0.0107	1.07	
		C	1				129.39	13769.03	0.0094	0.94	0.99 +/- 0.05 %
			2				228.81	21856.52	0.0105	1.05	
			3				134.35	13644.69	0.0098	0.98	
	58	A	1				124.52	15177.10	0.0082	0.82	0.83 +/- 0.22 %
			2				164.31	15500.34	0.0106	1.06	
			3				85.63	13802.38	0.0062	0.62	
		B	1				139.55	14851.93	0.0094	0.94	1.04 +/- 0.13 %
			2				172.84	17413.33	0.0099	0.99	
			3				175.90	14733.52	0.0119	1.19	
		C	1				163.45	16740.57	0.0098	0.98	0.86 +/- 0.16 %
			2				128.52	18713.29	0.0069	0.69	
			3				151.45	16280.46	0.0093	0.93	
N10	59	A	1	Mini-Pig	1867	25-Aug-09	58.72	16660.78	0.0035	0.35	0.34 +/- 0.10 %
			2				37.38	15727.48	0.0024	0.24	
			3				55.98	12826.20	0.0044	0.44	
		B	1				18.15	14622.94	0.0012	0.12	0.26 +/- 0.26 %
			2				14.99	14084.67	0.0011	0.11	
			3				74.29	13327.46	0.0056	0.56	
		C	1				112.32	24679.40	0.0046	0.46	0.65 +/- 0.19 %
			2				138.75	16858.04	0.0082	0.82	
			3				101.78	14867.33	0.0068	0.68	
	60	A	1				108.97	17199.64	0.0063	0.63	0.39 +/- 0.21 %
			2				41.67	13143.07	0.0032	0.32	
			3				27.44	11816.06	0.0023	0.23	
		B	1				120.28	12293.92	0.0098	0.98	0.72 +/- 0.23 %
			2				74.95	13869.91	0.0054	0.54	
			3				80.21	12414.19	0.0065	0.65	
		C	1				180.37	15252.91	0.0118	1.18	1.39 +/- 0.21 %
			2				229.99	14372.14	0.0160	1.60	
			3				203.68	14564.65	0.0140	1.40	

Melanin Content Measurements for the Yorkshire-Pig:

RHDV	Slide #	Slide Section	Area	Swine	Subject #	Sample date	Melanin area, A_M (μm^2)	Epidermis area, A_E (μm^2)	Melanin content index = A_M/A_E	Melanin (% epidermal area)	Avg melanin % for sample +/- standard deviation
N09	236	A	1	Yorkshire	3	15-May-09	86.19	13897.34	0.0062	0.62	0.53 +/- 0.27 %
			2				30.66	13175.02	0.0023	0.23	

			3				81.52	11010.69	0.0074	0.74	
		B	1				21.65	16748.15	0.0013	0.13	0.14 +/- 0.01%
			2				18.16	12489.45	0.0015	0.15	
			3				17.56	12005.77	0.0015	0.15	
		C	1				41.27	13221.16	0.0031	0.31	0.20 +/- 0.13 %
			2				6.85	13973.25	0.0005	0.05	
			3				32.16	13867.59	0.0023	0.23	
		D	1				20.35	24103.52	0.0008	0.08	0.13 +/- 0.04 %
			2				35.55	22938.21	0.0015	0.15	
			3				25.61	17078.97	0.0015	0.15	
		E	1				63.33	19388.07	0.0033	0.33	0.21 +/- 0.11 %
			2				35.31	17469.61	0.0020	0.20	
			3				19.66	19861.84	0.0010	0.10	
		F	1				63.95	10422.98	0.0061	0.61	0.54 +/- 0.23 %
			2				35.59	12903.27	0.0028	0.28	
			3				69.92	9755.09	0.0072	0.72	
N09	237	A	1	Yorkshire	4	13-May-09	82.56	15936.69	0.0052	0.52	0.36 +/- 0.30 %
			2				81.53	14927.61	0.0055	0.55	
			3				4.29	19968.97	0.0002	0.02	
		B	1				21.57	12141.49	0.0018	0.18	0.17 +/- 0.11 %
			2				9.07	15996.81	0.0006	0.06	
			3				44.30	16186.91	0.0027	0.27	
		C	1				36.24	14923.33	0.0024	0.24	0.29 +/- 0.13 %
			2				72.07	16285.20	0.0044	0.44	
			3				31.04	15830.23	0.0020	0.20	
		D	1				76.51	14509.86	0.0053	0.53	0.46 +/- 0.07 %
			2				53.43	13513.28	0.0040	0.40	
			3				64.84	14027.85	0.0046	0.46	
		E	1				54.88	16669.41	0.0033	0.33	0.29 +/- 0.04 %
			2				85.46	30176.88	0.0028	0.28	
			3				54.99	21884.42	0.0025	0.25	
		F	1				153.93	15146.73	0.0102	1.02	0.67 +/- 0.31 %
			2				78.19	14480.90	0.0054	0.54	
			3				72.96	16571.28	0.0044	0.44	

RHDV	Slide #	Slide Section	Area	Swine	Subject #	Sample date	Melanin area, A_M (μm^2)	Epidermis area, A_E (μm^2)	Melanin content index = A_M/A_E	Melanin (% epidermal area)	Avg melanin % for sample +/- std dev
N09	238	A	1	Yorkshire	5	13-May-09	77.23	17787.33	0.0043	0.43	0.48 +/- 0.21 %
			2				45.58	15275.87	0.0030	0.30	
			3				114.18	16191.59	0.0071	0.71	
		B	1				29.08	11145.10	0.0026	0.26	0.47 +/- 0.18 %
			2				102.55	18179.54	0.0056	0.56	

			3				62.25	10823.70	0.0058	0.58	
		C	1				74.54	14476.60	0.0051	0.51	0.37 +/- 0.12 %
			2				65.56	21127.21	0.0031	0.31	
			3				50.64	17074.55	0.0030	0.30	
		D	1				97.81	13336.61	0.0073	0.73	0.50 +/- 0.22 %
			2				31.92	11077.76	0.0029	0.29	
			3				57.48	11803.10	0.0049	0.49	
		E	1				79.10	17963.51	0.0044	0.44	0.39 +/- 0.05 %
			2				50.66	14698.15	0.0034	0.34	
			3				46.51	12208.80	0.0038	0.38	
		F	1				69.56	16503.89	0.0042	0.42	0.44 +/- 0.03 %
			2				115.03	27343.50	0.0042	0.42	
			3				111.65	23687.20	0.0047	0.47	
N09	239	A	1	Yorkshire	170	7-May-09	49.04	16210.91	0.0030	0.30	0.72 +/- 0.41 %
			2				211.25	18910.02	0.0112	1.12	
			3				168.62	22525.40	0.0075	0.75	
		B	1				73.79	24689.44	0.0030	0.30	0.27 +/- 0.12 %
			2				37.72	10236.66	0.0037	0.37	
			3				16.21	11587.61	0.0014	0.14	
		C	1				84.20	12176.37	0.0069	0.69	0.62 +/- 0.23 %
			2				131.04	16209.33	0.0081	0.81	
			3				51.16	14277.15	0.0036	0.36	
		D	1				115.79	13953.47	0.0083	0.83	0.77 +/- 0.15 %
			2				129.77	14588.22	0.0089	0.89	
			3				84.20	13973.30	0.0060	0.60	
		E	1				18.28	22690.51	0.0008	0.08	0.52 +/- 0.38 %
			2				166.87	22047.19	0.0076	0.76	
			3				149.28	20862.14	0.0072	0.72	
		F	1				34.75	15348.19	0.0023	0.23	0.40 +/- 0.22 %
			2				57.53	18238.41	0.0032	0.32	
			3				58.82	9105.16	0.0065	0.65	

RHDV	Slide #	Slide Section	Area	Swine	Subject #	Sample date	Melanin area, A_M (μm^2)	Epidermis area, A_E (μm^2)	Melanin content index = A_M/A_E	Melanin (% epidermal area)	Avg melanin % for sample +/- std dev
N09	240	A	1	Yorkshire	172	7-May-09	258.84	21027.66	0.0123	1.23	1.30 +/- 0.10 %
			2				232.91	16390.07	0.0142	1.42	
			3				174.97	13984.46	0.0125	1.25	
		B	1				233.59	16446.09	0.0142	1.42	1.72 +/- 0.72 %
			2				146.12	12095.23	0.0121	1.21	
			3				488.25	19200.15	0.0254	2.54	
		C	1				109.73	13856.88	0.0079	0.79	0.41 +/- 0.34 %
			2				43.66	15598.97	0.0028	0.28	

			3				28.97	17983.81	0.0016	0.16	
		D	1				165.17	14709.05	0.0112	1.12	0.46 +/- 0.57 %
			2				34.22	19928.24	0.0017	0.17	
			3				12.12	12636.00	0.0010	0.10	
		E	1				283.16	26042.44	0.0109	1.09	1.35 +/- 0.40%
			2				241.24	20774.98	0.0116	1.16	
			3				309.56	17142.50	0.0181	1.81	
		F	1				40.71	22519.04	0.0018	0.18	0.13 +/- 0.05 %
			2				12.34	11444.34	0.0011	0.11	
			3				17.91	19213.16	0.0009	0.09	
N09	241	A	1	Yorkshire	173	6-May-09	54.47	13194.73	0.0041	0.41	0.24 +/- 0.15 %
			2				26.89	18505.36	0.0015	0.15	
			3				31.09	19115.55	0.0016	0.16	
		B	1				67.08	18469.29	0.0036	0.36	0.17 +/- 0.17 %
			2				24.03	27087.4	0.0009	0.09	
			3				10.81	18432.74	0.0006	0.06	
		C	1				69.63	14423.46	0.0048	0.48	0.45 +/- 0.10 %
			2				93.19	17571.02	0.0053	0.53	
			3				48.59	14364.54	0.0034	0.34	
		D	1				8.29	10512.09	0.0008	0.08	0.18 +/- 0.09 %
			2				37.39	18513.96	0.0020	0.20	
			3				32.59	12526.09	0.0026	0.26	
		E	1				33.29	14452.66	0.0023	0.23	0.18 +/- 0.05 %
			2				18.63	15143.59	0.0012	0.12	
			3				26.12	14506.43	0.0018	0.18	
		F	1				131.19	15447.02	0.0085	0.85	0.36 +/- 0.43 %
			2				12.34	17982.45	0.0007	0.07	
			3				19.23	12766.44	0.0015	0.15	

RHDV	Slide #	Slide Section	Area	Swine	Subject #	Sample date	Melanin area, A_M (μm^2)	Epidermis area, A_E (μm^2)	Melanin content index = A_M/A_E	Melanin (% epidermal area)	Avg melanin % for sample +/- std dev
N09	242	A	1	Yorkshire	171	6-May-09	52.68	13777.65	0.0038	0.38	0.36 +/- 0.14 %
			2				69.67	14123.93	0.0049	0.49	
			3				28.63	13823.91	0.0021	0.21	
		B	1				43.32	18622.78	0.0023	0.23	0.22 +/- 0.06 %
			2				28.86	10633.46	0.0027	0.27	
			3				25.04	15743.20	0.0016	0.16	
		C	1				87.96	16313.66	0.0054	0.54	0.49 +/- 0.18 %
			2				98.99	15715.52	0.0063	0.63	
			3				38.52	13446.63	0.0029	0.29	
		D	1				34.24	30177.23	0.0011	0.11	0.28 +/- 0.20 %
			2				40.30	18589.85	0.0022	0.22	

			3				78.03	15720.99	0.0050	0.50	
		E	1				100.35	13739.96	0.0073	0.73	0.47 +/- 0.23 %
			2				77.82	25756.91	0.0030	0.30	
			3				71.26	18514.06	0.0038	0.38	
		F	1				55.66	19535.18	0.0028	0.28	0.26 +/- 0.04 %
			2				107.17	37723.05	0.0028	0.28	
			3				55.48	25946.88	0.0021	0.21	
N09	243	A	1	Yorkshire	6	14-May-09	53.06	16788.31	0.0032	0.32	0.44 +/- 0.29 %
			2				53.27	22254.97	0.0024	0.24	
			3				116.30	15103.02	0.0077	0.77	
		B	1				40.75	17492.74	0.0023	0.23	0.12 +/- 0.10 %
			2				5.95	12785.82	0.0005	0.05	
			3				12.10	14248.01	0.0008	0.08	
		C	1				150.49	12978.12	0.0116	1.16	0.74 +/- 0.37 %
			2				88.15	15681.91	0.0056	0.56	
			3				89.83	18172.68	0.0049	0.49	
		D	1				144.61	20073.09	0.0072	0.72	0.38 +/- 0.30 %
			2				21.37	13423.94	0.0016	0.16	
			3				37.17	14436.74	0.0026	0.26	
		E	1				22.61	17929.63	0.0013	0.13	0.10 +/- 0.02 %
			2				16.87	16461.47	0.0010	0.10	
			3				24.51	29278.49	0.0008	0.08	
		F	1				34.25	17179.49	0.0020	0.20	0.26 +/- 0.11 %
			2				68.90	17981.92	0.0038	0.38	
			3				58.33	30037.22	0.0019	0.19	

Appendix 3: p-Values for Skin Thickness Measurements

F-Test: p-values ≤ 0.05 are considered to have statistically different variances.

Dermis	Micro-Pig	Mini-Pig (1857)	Mini-Pig (1867)	Yorkshire (3)	Yorkshire (4)	Yorkshire (5)	Yorkshire (170)	Yorkshire (172)	Yorkshire (173)	Yorkshire (171)	Yorkshire (6)
Micro-Pig											
Mini-Pig (1857)	0.0000										
Mini-Pig (1867)	0.0358	0.0213									
Yorkshire (3)	0.0000	0.1748	0.0003								
Yorkshire (4)	0.0000	0.0000	0.0000	0.0057							
Yorkshire (5)	0.0465	0.0159	0.9123	0.0002	0.0000						
Yorkshire (170)	0.0020	0.1922	0.3115	0.0083	0.0000	0.2620					
Yorkshire (172)	0.1867	0.0022	0.4309	0.0000	0.0000	0.4979	0.0729				
Yorkshire (173)	0.0031	0.1524	0.3766	0.0057	0.0000	0.3203	0.8979	0.0955			
Yorkshire (171)	0.6555	0.0000	0.0112	0.0000	0.0000	0.0152	0.0004	0.0780	0.0007		
Yorkshire (6)	0.0000	0.1647	0.0003	0.9739	0.0062	0.0002	0.0075	0.0000	0.0052	0.0000	

Epidermis	Micro-Pig	Mini-Pig (1857)	Mini-Pig (1867)	Yorkshire (3)	Yorkshire (4)	Yorkshire (5)	Yorkshire (170)	Yorkshire (172)	Yorkshire (173)	Yorkshire (171)	Yorkshire (6)
Micro-Pig											
Mini-Pig (1857)	0.0214										
Mini-Pig (1867)	0.2867	0.2117									
Yorkshire (3)	0.1053	0.0001	0.0077								
Yorkshire (4)	0.0162	0.0000	0.0006	0.4246							
Yorkshire (5)	0.8131	0.0385	0.4066	0.0638	0.0084						
Yorkshire (170)	0.0667	0.0000	0.0040	0.8284	0.5605	0.0388					
Yorkshire (172)	0.1674	0.3506	0.7512	0.0030	0.0002	0.2519	0.0015				
Yorkshire (173)	0.4745	0.0027	0.0759	0.3633	0.0887	0.3416	0.2606	0.0369			
Yorkshire (171)	0.0234	0.0000	0.0010	0.5106	0.8880	0.0125	0.6589	0.0003	0.1181		
Yorkshire (6)	0.0074	0.0000	0.0002	0.2803	0.7778	0.0036	0.3877	0.0001	0.0477	0.6723	

Corneum	Micro-Pig	Mini-Pig (1857)	Mini-Pig (1867)	Yorkshire (3)	Yorkshire (4)	Yorkshire (5)	Yorkshire (170)	Yorkshire (172)	Yorkshire (173)	Yorkshire (171)	Yorkshire (6)
Micro-Pig											
Mini-Pig (1857)	0.7899										
Mini-Pig (1867)	0.0233	0.0448									
Yorkshire (3)	0.0160	0.0317	0.8849								
Yorkshire (4)	0.0064	0.0137	0.6397	0.7463							
Yorkshire (5)	0.0479	0.0251	0.0000	0.0000	0.0000						
Yorkshire (170)	0.1281	0.2086	0.4488	0.3672	0.2211	0.0005					
Yorkshire (172)	0.2487	0.3744	0.2598	0.2039	0.1115	0.0019	0.7108				
Yorkshire (173)	0.0003	0.0001	0.0000	0.0000	0.0000	0.0933	0.0000	0.0000			
Yorkshire (171)	0.4615	0.6382	0.1229	0.0919	0.0450	0.0070	0.4301	0.6753	0.0000		
Yorkshire (6)	0.0406	0.0741	0.8225	0.7121	0.4888	0.0001	0.5938	0.3663	0.0000	0.1868	

Two-tailed, equal or unequal variances (dependent on F-test results) t-Test: p-values ≤ 0.05 are considered to have statistically different means.

Dermis	Micro-Pig	Mini-Pig (1857)	Mini-Pig (1867)	Yorkshire (3)	Yorkshire (4)	Yorkshire (5)	Yorkshire (170)	Yorkshire (172)	Yorkshire (173)	Yorkshire (171)	Yorkshire (6)
Micro-Pig											
Mini-Pig (1857)	0.0016										
Mini-Pig (1867)	0.0000	0.0668									
Yorkshire (3)	0.0000	0.0000	0.0001								
Yorkshire (4)	0.0182	0.0001	0.0000	0.0000							
Yorkshire (5)	0.0899	0.0000	0.0000	0.0000	0.1373						
Yorkshire (170)	0.1191	0.1056	0.0001	0.0000	0.0025	0.0053					
Yorkshire (172)	0.1366	0.0467	0.0000	0.0000	0.0029	0.0043	0.7901				
Yorkshire (173)	0.5755	0.0164	0.0000	0.0000	0.0115	0.0555	0.3859	0.4865			
Yorkshire (171)	0.0000	0.0001	0.0073	0.0126	0.0000	0.0000	0.0000	0.0000	0.0000		
Yorkshire (6)	0.0009	0.5973	0.2986	0.0000	0.0000	0.0000	0.0460	0.0201	0.0073	0.0034	

Epidermis	Micro-Pig	Mini-Pig (1857)	Mini-Pig (1867)	Yorkshire (3)	Yorkshire (4)	Yorkshire (5)	Yorkshire (170)	Yorkshire (172)	Yorkshire (173)	Yorkshire (171)	Yorkshire (6)
Micro-Pig											
Mini-Pig (1857)	0.9255										
Mini-Pig (1867)	0.7434	0.7807									
Yorkshire (3)	0.2258	0.1573	0.1194								
Yorkshire (4)	0.0777	0.0468	0.0353	0.5567							
Yorkshire (5)	0.1778	0.1029	0.0771	0.9754	0.4999						
Yorkshire (170)	0.0950	0.0565	0.0426	0.6581	0.8722	0.6023					
Yorkshire (172)	0.3395	0.3115	0.5007	0.0351	0.0091	0.0156	0.0102				
Yorkshire (173)	0.2626	0.1795	0.1353	0.8730	0.4427	0.8828	0.5325	0.0364			
Yorkshire (171)	0.0351	0.0181	0.0139	0.3703	0.7732	0.3100	0.6444	0.0030	0.2742		
Yorkshire (6)	0.0014	0.0005	0.0004	0.0483	0.1790	0.0291	0.1218	0.0001	0.0271	0.2822	

Corneum	Micro-Pig	Mini-Pig (1857)	Mini-Pig (1867)	Yorkshire (3)	Yorkshire (4)	Yorkshire (5)	Yorkshire (170)	Yorkshire (172)	Yorkshire (173)	Yorkshire (171)	Yorkshire (6)
Micro-Pig											
Mini-Pig (1857)	0.0347										
Mini-Pig (1867)	0.5714	0.0195									
Yorkshire (3)	0.0006	0.0000	0.0092								
Yorkshire (4)	0.0000	0.0000	0.0005	0.3201							
Yorkshire (5)	0.4344	0.1093	0.2139	0.0000	0.0000						
Yorkshire (170)	0.0004	0.0000	0.0095	0.8685	0.2268	0.0000					
Yorkshire (172)	0.0925	0.0004	0.3564	0.0577	0.0039	0.0115	0.0647				
Yorkshire (173)	0.2978	0.0006	0.8518	0.0020	0.0000	0.0265	0.0014	0.3009			
Yorkshire (171)	0.0003	0.0000	0.0099	0.7607	0.1661	0.0000	0.8878	0.0716	0.0010		
Yorkshire (6)	0.1575	0.0015	0.4605	0.0532	0.0041	0.0306	0.0598	0.8835	0.4440	0.0664	

GLOSSARY

AFRL – Air Force Research Laboratory

F-M - Fontana-Masson stain used in histology to examine melanin

H&E – Hematoxylin and eosin stain commonly used in histology

Melanin Index – The ratio of the area occupied by melanin to the total area of a selected area of tissue as selected in a ROI

RHDO – The four-letter branch under AFRL in the 711th Human Performance Wing, Human Effectiveness Directorate, Directed Energy Bioeffects Division which investigates Optical Radiation

ROI – Region of interest, as in an area selected in an image by a computer program for analysis

Steiner Stain – A stain used in histology

UV – Ultraviolet, a region in the electromagnetic spectrum